AD_____

Award Number DAMD17-98-1-8026

TITLE: The Role of Steroid Receptor Coactivator-1 in Breast Cancer

PRINCIPAL INVESTIGATOR: Neil J. McKenna, Ph.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, Texas 77030

REPORT DATE: June 1999

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

DTIC QUALITY INSPECTED 4

20000828 178

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 1999	Annual Summary (18 May 98 - 17 May 99)

4. TITLE AND SUBTITLE
The Role of Steroid Receptor Coactivator-1 in Breast Cancer

5. FUNDING NUMBERS
DAMD17-98-1-8026

6. AUTHOR(S)
Neil J. McKenna, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor College of Medicine
Houston, Texas 77030

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The steroid hormones estrogen and progesterone and their intracellular receptors have been implicated in the development and progression of primary epithelial breast cancer. Regulation of gene expression by hormone-bound steroid receptors is mediated by members of the steroid receptor coactivator (SRC) family. The purpose of this study was to examine the role of SRC-1 in the regulation of gene expression by steroid receptors in breast cancer. SRC-1 was found to exist in a multifactor complex which was stably associated with liganded progesterone receptor, implicating an active SRC-1 complex as a primary target of liganded PR in breast cancer cells. In an effort to further define the role of SRC-1 in breast cancer, we characterized a member of the active SRC-1 complex, SRA (steroid receptor RNA coactivator), which was shown to function as an RNA. Significantly, SRA is a limiting factor required for efficient transcriptional activation by steroid hormone receptors in breast cancer cells. Furthermore, it exhibits a specific pattern of overexpression in breast cancer cell lines and primary breast tumors, suggesting that the active SRC-1 complex may be a critical factor in the development of certain types of breast cancer. The characterization of other members of the active SRC-1 complex in breast cancer is currently being pursued.

14. SUBJECT TERMS
Breast Cancer, steroid receptor coactivators, steroid hormone receptors, steroid hormones, gene expression

15. NUMBER OF PAGES
55

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

__✓__ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

__✓__ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

__✓__ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

__✓__ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

__✓__ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature 6/21/99 Date

The Role of Steroid Receptor Coactivator-1 in Breast Cancer

Annual Summary Report, 1998-1999

Table of contents

Introduction

Steroid hormones have a historically well-characterized role in the development and progression of primary epithelial breast cancer. Their physiological effects are known to be mediated by intracellular receptors which bind their cognate hormones and specifically regulate the expression of genes in the nucleus of their target cells, which include those of the mammary epithelium. Recently, a growing number of factors has been identified which experimental evidence has implicated as key intermediary components of steroid hormone receptor action. Referred to as coactivators, these multifunctional molecules are recruited by liganded receptors to create a transcriptionally permissive environment at the promoters of their target genes, culminating in the finely regulated expression of these genes. Currently, key questions in the study of steroid-dependent breast cancer focus upon (i) the exact mode of action of steroid receptor coactivators and (ii) their role at the molecular level in the genesis and progression of these tumors. Perhaps the best characterized family of steroid receptor coactivators is the SRC family, named after the initial cloning of steroid receptor coactivator-1 and now known to include two other members, SRC-2 and SRC-3.

My purpose is to examine the role of SRC-1 at the molecular level in breast cancer. The remarkable growth rate of studies on the SRC family has emphasized the complexity of the relationships between individual coactivators and their cognate receptors. Consequently, we believe that a clear picture of the functional role of a single coactivator in a disease as intractable as breast cancer will not emerge without a global perspective on other members of the family. For example, targeted deletion of the SRC-1 gene in mice results in a compensatory overexpression of another family member of SRC-2, a probable contributing factor in the unanticipated stable phenotype of the SRC-1 null mutant mouse. In addition, as I will describe, these molecules exist not in isolation, but as members of active multiprotein complexes, and any consideration of their role in breast cancer must embrace other members of these complexes. For this reason, the scope of the project encompasses the role of SRC-1 and other members of the SRC family, in addition to factors characterized as members of SRC complexes.

Body

After submission of the original proposal, a third member of the SRC family, SRC-3 was cloned. We were interested in the potential role of SRC-3 as a key factor in the development of breast cancer, particularly given (i) its different functional specificity with respect to either of the other two members of this family and (ii) its pattern of overexpression in 64% of breast tumors analyzed in a recent study. An obvious question was whether this pattern of overexpression was a cause or an effect of the growth of these tumors. For this reason, studies were initiated in our laboratory to investigate the effect of overexpression in mice of a mammary transgene encoding SRC-3. We are awaiting the generation of a mouse strain overexpressing SRC-3, and anticipate that these studies will yield valuable insights into the role of SRC family members in breast cancer.

During the past year, we have been attempting to discern the mechanism whereby SRC-1 exerts its effects on steroid receptor regulation of gene expression in breast cancer cells. Fundamental questions are (i) does SRC-1 act in isolation or are its molecular effects exerted as part of an active multifactor complex, (ii) if so, what is the nature of this complex and its relationship with steroid receptors, and (iii) what is the role of these complexes in the development of breast cancer? Our initial studies pursued the hypothesis that SRC-1 was present in the cell not as a single molecule but as a multifactor complex in breast cancer cells. The breast cancer cell line T47D was chosen for this part of the study since it is cytologically well-characterized, contains functional progesterone receptor and is routinely cultured in our department. T47D cell lysates were subjected to biochemical fractionation and screened by immunoblotting using coregulator-specific antibodies. SRC-1 was observed as a component of multiprotein complexes of an estimated 600-700-kDa in size, much greater than its molecular weight of 160-kDa. Significantly, we showed that these complexes may be functionally important in breast cancer since they were recruited by PR in the presence of its ligand, progesterone, in breast cancer cells. This was the first direct evidence that SRC-1 interacted with steroid receptors as part of an active multifactor signaling complex in breast cancer cells.

Having established that SRC-1 is present in a stable complex with other factors in breast cancer cells, we hypothesized that factors contained in the SRC-1 complex are potentially important therapeutic targets, and that their characterization would be a natural extension of the research proposed in

the statement of work. We chose to examine the cellular factors present in the active SRC-1 complex in breast cancer cell lines, since these may be important factors in the etiology of breast cancer. The next section describes the characterization of one of these SRC-1 associated factors and its potentially exciting role as a diagnostic and/or prognostic marker in primary breast cancer.

SRA: an RNA member of the active SRC-1 complex

Because factors that associate with liganded steroid receptors as part of a complex with SRC-1 might represent novel therapeutic targets in breast cancer, we wanted to characterize the factors present in the SRC-1 complex. The laboratory of my mentor, Dr. Bert W. O'Malley, identified a clone SRA (for steroid receptor RNA activator) which interacted with the human PR. When tested in transient transfection with nuclear receptors this clone specifically enhanced transactivation by steroid (type I) receptors rather than type II nuclear receptors. This was of particular interest since many breast tumors are specifically steroid-dependent and do not require ligands for type II receptors for their growth and progression to malignancy. Further work suggested that this clone did not function as a protein since frameshift and stop codon insertions, which would result in a mistranslated, non-functional protein, had no effect on the coactivation potential of this molecule in transient transfections. Finally, transfection experiments in the presence of cycloheximide, an inhibitor of *de novo* protein synthesis, indicated that SRA did not require protein synthesis to enhance transcription of a reporter gene. We concluded that the factor acted as an RNA, and referred to it as SRA, for steroid receptor RNA activator (Lanz *et al.* 1999).

Given the functional specificity of SRA as a steroid receptor-selective coactivator, we hypothesized that SRA might be a member of the functionally active SRC-1 complex in breast cancer cells. To investigate SRC-1/SRA interactions, we fractionated cell extracts from T47D human breast cancer cell lysates on a Superose 6 column as previously described. One half of the collected fractions was processed for western analysis using a specific antibody against SRC-1 and the remainder of each fraction was subjected to RNA isolation followed by SRA transcript-specific RT-PCR and Southern analysis. SRC-1 and SRA were detected in a specific complex of 600-700 kDa. Further work showed that this complex was recruited by a liganded steroid receptor, a strong indication that the regulation of gene expression by

steroid hormone receptors in breast cancer cells requires an active complex containing SRC-1 in addition to an RNA factor, SRA.

Significance of SRA in breast cancer

Three lines of evidence suggested that the SRC-1-associated factor SRA might be a critical factor in the development of breast cancer.

1. The steroid hormones estrogen and progesterone have a well-characterized role in the development and progression of primary epithelial breast cancer When used in transient transfection of breast cancer cells we found that SRA was able to enhance the transactivation potential of the estrogen receptor (ER) and the progesterone receptor (PR), suggesting that it may mediate the regulation of gene expression by estrogen and progesterone in breast cancer cells *in vivo*.
2. SRA exerts its coactivation by interaction with activation function 1 (AF-1) of steroid receptors, including ER. The AF-1 of the ER is not affected by treatment with the AF-2 inhibitor tamoxifen, suggesting that as an AF-1 cofactor, SRA might be a factor in the tamoxifen-insensitive growth commonly observed in certain advanced forms of breast cancer.
3. Thirdly, we used Northern analysis to examine the expression of the SRA isoforms in breast cancer cells. SRA is expressed as a number of different isoforms, one of which is specifically expressed in the human breast cancer cell lines MCF-7 and T47D. Expression of this isoform was observed in no other cell line studied, indicating that it may have a functionally relevant role in the etiology of breast cancer. Our results suggest that SRA has potential for prognostic evaluation in breast cancer. Work is currently in hand to assess the clinical expression pattern of SRA in breast tumor samples. A marked association of SRA overexpression in primary breast tumors has been noted in preliminary studies (data unpublished). As a natural extension of the research proposed in the statement of work, I am particularly interested in correlating the expression of SRC-1 with that of SRA. Our hypothesis is that SRA, as a member of an active SRC-1 complex will have an important functional role in the regulation of gene expression by steroid hormone receptors in steroid-dependent tumors.

Other members of the active SRC-1 complex in breast cancer cells

Currently I am attempting to characterize other members of the SRC-1 complex, using a variety of biochemical approaches in breast cancer nuclear lysates. I have identified a complex of 8-10 proteins which appears to associate specifically with a full length progesterone receptor in the presence of ligand (data unpublished). Work is currently in hand to identify these proteins and attain a level of purification which will enable direct protein sequencing of these factors. We anticipate that these proteins will have an important impact on our knowledge of the role of the SRC-1 complex in the development of breast cancer.

Design and construction of vectors for overexpression of full-length SRC-1 (SRC-1 FL)

Vectors for overexpression of SRC-1FL were successfully designed and cloned. The vector pIRES-Neo (Clontech) was chosen since it permits stable episomal expression of SRC-1 in the cell. In addition, the bicistronic design of this vector ensures that the expression of the protein of interest is tightly coupled to that of the selective marker. The SRC-1 was tagged using a FLAG sequence enabling specific detection of SRC-1 in nuclear extracts by the highly sensitive FLAG antibody. HeLa cells are currently being used to determine the expression efficiency of pIRES-neo-SRC-1 due to the fact that they are readily transfected and hence eliminate transfection efficiency as a variable. Many factors influence expression of the tagged SRC-1 protein, including stability of the bicistronic mRNA, proteolytic sensitivity of the fusion protein and toxicity of its overexpression in the host cell line. We have encountered some problems arising from contamination of clones by bacteria, and are currently working around these problems. Once clones are obtained, they will be used (i) to establish the effect of SRC-1 overexpression on cell growth and (ii) as an alternative source of SRC-1, if required, for analysis and characterization of the active SRC-1 complex.

Difficulties encountered in SRC-1 characterization in breast tumors

Degradation of SRC-1 has been a common problem in our efforts to establish the true value of SRC-1 as a prognostic marker in human breast tumors. SRC-1 has proven to be an unusually proteolytically sensitive protein, typically degrading into a prominent band at 140-kDa and another at 120-kDa. These bands were initially thought to represent individual isoforms of the full

length protein, but it may well be that they are merely proteolytic artifacts. The tumor or biopsy samples obtained from clinicians spend variable lengths of time between removal from the patient and snap-freezing in liquid nitrogen, rendering them differentially sensitive to proteolysis by proteases present in the tumor sample. As a consequence of these patterns of proteolysis we have been unable to determine real expression levels of SRC-1 relative to other cellular proteins. Hsp90 is both much more resistant to proteolysis than SRC-1 and a much more abundant protein, and is therefore not a suitable loading control for total protein. Until these difficulties are resolved we will be unable to determine the prognostic value of patterns of SRC-1 protein expression in primary breast cancer. We feel that ultimately analysis of SRC-1 expression using the SRC-1 antibody is a preferable approach to that of analyzing SRC-1 expression at the RNA level, using Northern or ribonuclease protection analysis. Overexpression of RNA, or sequence aberrations at the RNA level, do not necessarily reflect similar events at the protein level, preventing meaningful interpretations of results. In addition, RNA quality is similarly compromised after removal of tumor tissue from the patient.

Training as a scientific communicator

A fundamental component in the development of my career as a breast cancer researcher is my training as a scientific communicator. This will be of particular importance given the fact that, as a principal investigator, the authorship and review of research proposals and scientific manuscripts will be a daily routine. The rapid pace of progress in the area of steroid receptor coactivators over the past few years prompted me to take the opportunity to draw these disparate lines of research into a single, complete review of the field. In order to reach the largest possible audience in the field, we chose the journal *Endocrine Reviews*. This journal has the highest Impact Factor ranking of the 73 journals in the ISI (Institute of Scientific Information) category of endocrinology and metabolism. Of the total 4,730 scientific journals surveyed by ISI, *Endocrine Reviews'* Impact Factor ranking is #11 and its Immediacy Index ranking is #108. This article is likely to have a significant impact within the field and its preparation was a valuable opportunity to develop my skills as a scientific communicator, skills which will be of great value in my career as a breast cancer researcher.

Appendices to the summary

Key research accomplishments

- SRC-1 shown to function as a member of an active, multifactor complex in breast cancer cells.
- Active SRC-1 complex shown to be a primary target of hormone-bound steroid receptor in breast cancer cells.
- SRA, a steroid receptor coactivator, identified and characterized as a functional RNA transcript.
- SRA shown to be a member of the functional SRC-1 complex.
- Isoform of SRA shown to specifically overexpressed in breast cancer cell lines.
- SRA overexpressed in certain breast tumors (unpublished).
- Other members of the active SRC-1 complex being characterized (unpublished).

Reportable outcomes

McKenna, N.J., Lanz, R.B. and O'Malley, B.W. (1999) Nuclear receptor coregulators: cellular and molecular biology. *Endocrine Rev.* 20, 321-344.

Lanz, R.B., McKenna, N.J., Onate, S.A., Albrecht, U., Tsai, S.Y., Tsai, M.-J. and O'Malley, B.W. (1999). A novel steroid receptor coactivator, SRA, functions as an RNA transcript and is associated with SRC-1. OR1-1, 81st Annual Meeting of the Endocrine Society, San Diego, CA, USA (Abstr.).

Lanz, R.B., McKenna, N.J., Onate, S., Albrecht, U., Wong, J., Tsai, S.Y., Tsai, M.-J. and O'Malley, B.W. (1999) A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. *Cell* 97, 17-27.

McKenna, N.J., Xu, J., Nawaz, Z., Tsai, S.Y., Tsai, M.-J. and O'Malley, B.W. (1999) Nuclear receptor coactivators: multiple enzymes, multiple complexes, multiple functions. *J. Steroid Biochem. Mol. Biol.* 69, 3–12.

McKenna, N.J., Nawaz, Z., Tsai, S.Y., Tsai, M.-J. and O'Malley, B.W. (1998) Distinct steady state nuclear receptor coregulator complexes exist *in vivo*. *Proc. Natl. Acad. Sci. USA.* 95, 11697–11702.

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 11697–11702, September 1998
Cell Biology

Distinct steady-state nuclear receptor coregulator complexes exist *in vivo*

NEIL J. MCKENNA, ZAFAR NAWAZ, SOPHIA Y. TSAI, MING-JER TSAI, AND BERT W. O'MALLEY*

Department of Cell Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

Contributed by Bert W. O'Malley, August 7, 1998

ABSTRACT **Transcriptional regulation by members of the nuclear hormone receptor superfamily is a modular process requiring the mediation of distinct subclasses of coregulators. These subclasses include members of the steroid receptor coactivator-1 (SRC-1) coactivator family, p300/CBP and their associated proteins, such as p300/CBP-associated factor, human homologs of SWI/SNF proteins such as BRG-1, and the less well-characterized E3 ubiquitin-protein ligases E6 papillomavirus protein-associated protein and receptor-potentiating factor-1. Because functional studies indicate that these coregulators may form higher order complexes, we analyzed steady-state complexes of different coregulator subclasses *in vivo*. T47D and HeLa cell lysates were subjected to biochemical fractionation and screened by immunoblotting using coregulator-specific antibodies. We show that different subclasses of nuclear receptor coregulators exhibit distinct fractionation profiles. Furthermore, evidence is provided that SRC-1 family members may exist *in vivo* in heteromultimeric forms with each other. In addition, we demonstrate that liganded PR is present in stable complexes containing SRC-1 and transcription intermediary factor 2 (TIF2) *in vivo*. Our results suggest that the assembly of large, modular transcriptional complexes by recruitment of distinct subclasses of preformed coregulator subcomplexes may be involved in transcriptional regulation by activated nuclear receptors.**

Members of the nuclear receptor family of ligand-inducible transcription factors activate transcription in response to their ligands via enhancer elements located in the promoters of target genes (1). Recently it has become clear that transactivation by these receptors is a modular process, requiring interaction with an array of cofactors capable of (*i*) modifying the chromatin structure of hormone-regulated promoters by intrinsic histone acetyltransferase (HAT) activities, (*ii*) mediating interactions between the receptors and other transcription factors, and (*iii*) directing assembly and stabilization of the transcriptional preinitiation complex. Several structurally distinct subclasses of nuclear receptor coregulators have been identified, including: members of the steroid receptor coactivator-1 (SRC-1) family, the cointegrators p300 and CBP and their associated proteins; mammalian homologs of yeast SWI/SNF proteins; and the less well characterized E3 ubiquitin-protein ligase coactivators.

Our laboratory initially cloned SRC-1 as a factor required for transactivation by nuclear receptors (2), and SRC-1 has been termed variously as p160/NCoA-1 (3), and ERAP-160 (4). The subsequent identification of two more members of the SRC-1 family, namely transcription intermediary factor-2 [TIF2/GRIP-1/SRC-2] (5–7), and p/CIP (8) [ACTR (9)/RAC-3 (10)/AIB-1 (11)/TRAM-1 (12)/SRC-3] established the existence of a class of structurally and functionally related nuclear receptor coactivators. Sequence alignment of the members of the SRC-1 family highlights the shared domain structure throughout and predicts common modes of action by the individual members. SRC-1 family members have C-terminal domains that contain HAT activity, suggesting that they modify chromatin (9, 13). The presence in their extreme N termini of a postulated multimerization motif, the Per-Arnt Sim/basic helix–loop–helix homology domain (14), implies that molecular interactions between SRC-1 family members and other Per-Arnt-Sim/basic helix–loop–helix homology domain proteins might be important for their function *in vivo*.

A class of coregulators structurally distinct from the SRC-1 family, the cointegrators, is defined by the functionally related proteins p300 and CBP. These proteins exhibit broad functional specificity in addition to extensive amino acid sequence identity (15, 16) and are proposed to function by adapting signaling pathways and integrating stimuli into an appropriate transcriptional response at a wide variety of promoters (3, 17). CBP synergizes with SRC-1 in the potentiation of estrogen receptor and progesterone receptor (PR)-dependent transactivation (18), indicating a role in nuclear receptor-dependent signaling. In addition, p300/CBP were among the first regulators of mammalian transcription in which HAT activity was identified (19). Furthermore, proteins such as the SRC-1 family member p/CIP (8) and the HAT protein p300/CBP-associated factor (PCAF) (20), first identified as binding partners of p300/CBP, have been characterized as nuclear receptor-associated proteins and coregulators in their own right (21, 22).

The SWI proteins were first identified as potentially important intermediates in nuclear receptor action when yeast strains bearing mutations in *swi* genes were found to be incapable of supporting glucocorticoid receptor-dependent transactivation (23). Subsequently, human SWI/SNF homologs were found to enhance the activation functions of glucocorticoid receptor (24) as well as estrogen receptor and retinoic acid receptor (25), and it has been shown that glucocorticoid receptor directs ligand-dependent nucleosomal remodeling activity of the SWI/SNF complex in yeast (26). The mammalian homologs of the closely related yeast *swi2* and *snf2* genes are termed *brahma* and *brahma*-related gene-1 (*brg-1*), respectively. BRG-1, the product of the *brg-1* gene, has been shown to interact with glucocorticoid receptor in a ligand-dependent manner (27), suggesting that mammalian SWI/SNF proteins may be key elements in nuclear receptor action.

Another subclass of coregulators, relatively undefined functionally, but structurally distinct from those subclasses above, comprises the E3 ubiquitin-protein ligases receptor potentiat-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1998 by The National Academy of Sciences 0027-8424/98/9511697-6$2.00/0
PNAS is available online at www.pnas.org.

Abbreviations: SRC-1, steroid receptor coactivator-1; CBP, CREB-binding protein; BRG-1, product of the *brg*-1 gene; E6-AP, E6 papillomavirus protein-associated protein; RPF-1, receptor-potentiating factor-1; TIF2, transcription intermediary factor 2; PCAF, p300/CBP-associated factor; RNA pol II, RNA polymerase II; PR, progesterone receptor; HAT, histone acetytransferase.

*To whom reprint requests should be addressed. e-mail: berto@bcm.tmc.edu.

ing factor-1 (RPF-1) (28) and E6 papillomavirus protein-associated protein (E6-AP; Z.N., unpublished work). This subclass of coregulators differs from the SRC-1 family and the p300/CBP cointegrators in that they contain ubiquitin-protein ligase activity rather than HAT activity. They were initially identified as factors required for defining substrate specificity in proteolytic degradation by the proteosome system. The N-terminal receptor activation domains of E6-AP and RPF-1 are separable from their ubiquitin ligase domains that reside in their C-terminal HECT. In addition to these characterized subclasses of coregulators, a large number of receptor-interacting proteins have been identified, including RIP-140 (29), ARA-70 (30), Trip230 (31), and others.

Recently, attention has focused on mechanistic aspects of nuclear receptor coregulator function, in particular on the nature of the complexes that functional evidence indicates they potentially form. Liganded nuclear receptors are reported to recruit a variety of structurally diverse proteins: including SRC-1 family members SRC-1 (2), GRIP-1/TIF2/SRC-2 (5–7) and p/CIP/RAC3/AIB-1/ACTR/TRAM-1/SRC-3 (8–12); the cointegrators CBP and p300 (3, 32); PCAF (21, 22); human homologs of the yeast SWI/SNF proteins (27) as well as the E3 ubiquitin-protein ligase family members RPF-1 (28) and E6-AP (Z.N., unpublished work). In addition, multiple coregulator/coregulator interactions have been proposed, including p/CIP/CBP (8), CBP/PCAF (20), SRC-1/CBP (3), SRC-1/p300 (33), and SRC-1/PCAF (13). Viewed in their entirety, these individual observations raise questions concerning the steady-state organization of coregulators in the cell, as well as aspects of the nature, stability, and molecular relations of their putative complexes with activated nuclear receptors.

In light of these multiple reported interactions, we decided to address the steady-state relationships of multicoregulator transcriptional complexes *in vivo* by analyzing the biochemical fractionation profiles of coregulators representative of the different subclasses outlined above. We demonstrate that different subclasses of nuclear hormone receptor coregulators have distinct fractionation profiles. We suggest that at least two members of the SRC-1 coactivator family, SRC-1 and TIF2, can exist in stable complex(es) with each other *in vivo*. Furthermore, we provide evidence that PR interacts stably with complexes containing SRC-1 and TIF2 in a ligand-dependent manner. Our data suggest the existence of discrete, stable subcomplexes of different subclasses of coregulators that may facilitate the assembly of modular complexes required for transcriptional regulation by nuclear receptors.

MATERIALS AND METHODS

Cell Culture and Transient Transfections. Cell lines were routinely maintained at 37°C/5% CO_2 in DMEM (HeLa) or RPMI 1640 medium (T47D) supplemented with 5–10% charcoal-stripped fetal calf serum. Transfections were carried out using Lipofectin (Life Technologies, Gaithersburg, MD). pCR3.1-mCBP was constructed by inserting the *Bam*HI-*Bam*HI fragment of pRcRSV-mCBP8.0 into the corresponding site of pCR3.1 (Invitrogen). The construction of pCR3.1-E6-AP (Z.N., unpublished work), pCR3.1-hSRC-1A, and the reporter pPRE/GRE-E1b-Luc (21) have been described.

Gel Filtration. Subconfluent T47D or HeLa cells were washed and harvested in PBS and lysed with a disposable manual homogenizer in 50 mM NaCl/5 mM KCl/20 mM Hepes, pH 7.5/1 mM EDTA/10% glycerol containing a mixture of protease inhibitors (Sigma), and supplemented with ligand where appropriate. After centrifugation, the supernatant was loaded on a Superose 6 gel filtration column (Pharmacia) preequilibrated with 150 mM NaCl/50 mM sodium phosphate, pH 7.0 (supplemented with ligand where appropriate), and controlled by an FPLC system (Pharmacia). For antibody shift experiments, clarified lysates were rocked for 90 min at 4°C with 1–2 μg of SRC-1 antibody and a 3- to 4-fold excess of rabbit anti-mouse IgG (Zymed).

Immunoblotting. Immunoblotting was performed as described in Hanstein *et al.* (32). Commercially obtained antibodies used were anti-CBP (Upstate Biotechnologies, Lake Placid, NY), and anti-RNA polymerase II (RNA pol II) (Santa Cruz Biotechnology).

RESULTS

Subclasses of Nuclear Receptor Coregulators Exist in Primarily Distinct Complexes *in Vivo*. Our laboratory and others have previously shown that the functional interaction of nuclear hormone receptors with diverse subclasses of transcriptional coactivators is necessary for efficient receptor transactivation *in vivo* (2–3, 5–12, 27). Hypothesizing that such interactions might require the assembly of multiprotein complexes, we investigated the potential existence of nuclear hormone receptor coactivators in such complexes by biochemical fractionation of T47D and HeLa cell lysates, using a Superose 6 sizing column. Using antibodies against CBP and RNA pol II, we detected endogenous CBP and RNA pol II cofractionating in protein complexes of 1.5–2 MDa (Fig. 1), as estimated by Kee *et al.* (34). The elution profile of RNA pol II was much broader than that of CBP (Fig. 1; compare fractions 27–30 for CBP with fractions 26–34 for RNA pol II), also consistent with previous reports (34). We then compared the fractionation profile of endogenous CBP with that of purified baculovirus-expressed CBP, which elutes as an oligomer in distinct lower molecular size fractions (Fig. 1, CBP BAC fractions 31–36). This confirmed that CBP in T47D and HeLa cells forms high molecular weight multiprotein complexes *in vivo*, consistent with previous reports (34). In addition, the elution pattern of p300 in cell lysates closely resembled that of CBP, peaking in fractions containing complexes of 1.5–2 MDa, but more detectable in later fractions than CBP (Fig. 2, fractions 27–34).

We next compared the elution profiles of p300/CBP and RNA pol II with those of another class of nuclear receptor coregulators, the human homologs of the yeast SWI/SNF mediator complex proteins, which include BRG-1, the 220-kDa human homolog of yeast SWI2, and BAF-57, a 57 kDa-*B*RG-1-*a*ssociated *f*actor. These proteins exactly cofractionated in complexes of ≥2MDa (Fig. 2, peak fractions 25–27), consistent with previous estimates (35, 36). A distinct, second peak of BAF-57 was observed in later fractions (Fig. 2, peak fraction 38). Longer exposures of the BRG-1 immunoblots (data not shown) indicated that minor amounts of BRG-1

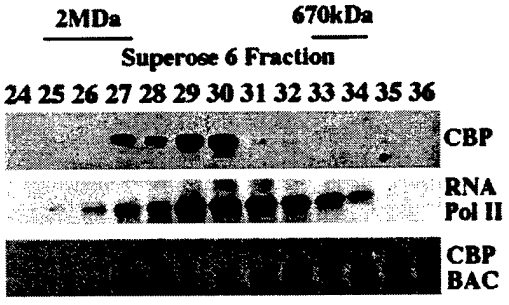

FIG. 1. High molecular mass complexes contain CBP and RNA pol II. Fractionation of T47D lysate on a Superose 6 column was analyzed by immunoblot with CBP and RNA Pol II-specific antibodies (CBP and RNA pol II). Recombinant baculovirus-expressed CBP also was fractionated (CBP BAC). Indicated are elution peaks of molecular mass markers: mammalian SWI/SNF complex (≈2 MDa) and thyroglobulin (670 kDa). The void volume (4 MDa for globular proteins) was determined at fraction 20 by silver staining after fractionation of T47D cell lysate (data not shown).

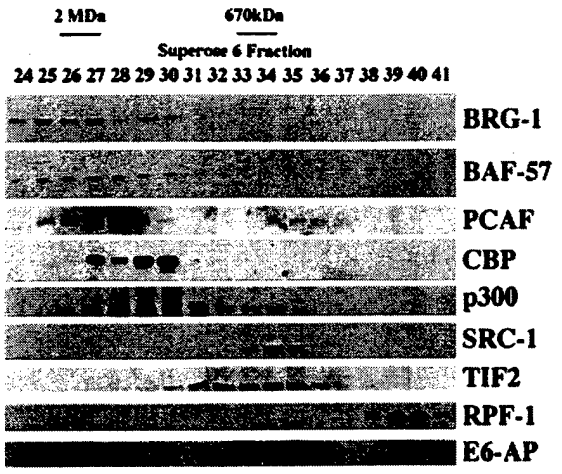

FIG. 2. Distinct steady-state fractionation profiles of different subclasses of nuclear receptor coregulators. T47D or HeLa cell lysate was fractionated on a Superose 6 column and subjected to immunoblot analysis by using coregulator-specific antibodies as indicated. Elution peaks of molecular mass standards are indicated. The relatively sharp elution peaks of SRC-1 and CBP were reproducible. No difference in fractionation pattern was observed between different cell lines.

copurified with the peak fractions of SRC-1 and TIF2 (see below).

In the light of previous reports of an interaction between the cointegrators p300/CBP and PCAF (20), we next examined whether PCAF peaked in the same fractions in which p300/CBP peaked. PCAF was found to peak slightly earlier than the elution peaks of p300/CBP (Fig. 2, peak fractions 27–28), indicating that PCAF is not an exclusive binding partner of p300 and may form complexes with other proteins such as the human homologs of the SWI/SNF complex. A second minor pool of PCAF was observed (Fig. 2, fractions 35–37), which may represent partially dissociated PCAF complexes, or complexes with factors other than p300/CBP. These PCAF pools were variable in proportion between runs (data not shown), and exhibited the greatest variation of all coregulators analyzed.

Because several studies have suggested that SRC-1 may exist in complexes with CBP (3) (Fig. 2, elution peak fractions 29–30), p300 (33) (Fig. 2, elution peak fractions 28–30) and PCAF (13) (Fig. 2, major elution peak fractions 27–28), we next analyzed the elution profile of SRC-1 in relation to these proteins. Analysis of the fractionation pattern of SRC-1 showed that it peaked sharply in fractions containing protein complexes of an estimated 0.5–0.6 MDa (Fig. 2, fractions 33–35). Overlap between the elution patterns of SRC-1 and CBP was undetectable (Fig. 2), implying that these proteins may exist in distinct preformed complexes, contrary to previous reports (3). In contrast, the elution pattern of SRC-1 overlapped slightly with minor pools of p300 and PCAF (Fig. 2), suggesting that should stable complexes between SRC-1 and these coregulators exist, they represent only a small proportion of their respective cellular pools.

Monomeric SRC-1 was undetectable in cell lysates, suggesting that the kinetics of the complex formation strongly favor the sequestration of SRC-1 in these complexes, or that the free form is subject to rapid degradation. As a control, we fractionated baculovirus-expressed SRC-1 by Superose 6 gel filtration and found that it eluted exclusively in fractions 32–35 (data not shown), similar to its elution profile in cell lysate (Fig. 2, lanes 33–35) that might indicate homomultimerization of SRC-1, but also may be attributable to incomplete purification of recombinant SRC-1 from insect cell coregulators. Similar to its elution profile in T47D and HeLa cell lysate, no monomeric purified SRC-1 was detectable, further suggesting that the free form of SRC-1 may be kinetically unstable. We then examined the elution profile of a second member of the SRC-1 family, TIF2. TIF2 copurified with SRC-1, although its elution pattern was less defined and covered a wider range of fractions than SRC-1 (Fig. 2, fractions 31–36). No cross reactivity was observed between the SRC-1 antibody and TIF2 in immunoblots (not shown). The relatively broad elution profile of TIF2 suggests that it might form a greater variety of complexes than its family member SRC-1.

These initial observations suggested to us that different subclasses of coactivator involved in nuclear receptor transactivation might be sequestered in largely distinct complexes. To further test this hypothesis, we examined the elution profiles of two members of a less well-defined but functionally distinct subclass of nuclear hormone coregulators, the E3 ubiquitin-protein ligases RPF-1 and E6-AP. E6-AP and RPF-1 proteins were observed to copurify in complexes of 200–300 kDa and are distinct from all of the complexes previously observed (Fig. 2, fractions 38–41).

E6-AP and RPF-1 Synergistically Enhance PR Transactivation. The copurification of E6-AP and RPF-1 by Superose gel filtration suggested to us that they might be present in common complexes. To test their possible functional interaction, we next examined whether these coactivators might synergistically enhance transactivation by PR. HeLa cells were transiently cotransfected with E6-AP/RPF-1, E6-AP/SRC-1, and E6-AP/CBP in a luciferase-based PR reporter assay (Fig. 3). Whereas the combinations of E6-AP/CBP (Fig. 3*a*) and E6-AP/SRC-1 (Fig. 3*b*) only additively enhanced PR transactivation, E6-AP and RPF-1 (Fig. 3*c*) synergistically enhanced PR transactivation.

Association of SRC-1 and TIF2 in a Single Complex *in Vivo*. While the copurification of SRC-1 and TIF2 was evidence that they might form a complex *in vivo* (Fig. 2), we verified this by incubating cell lysate with anti-SRC-1 antibody and rabbit anti-mouse IgG before fractionation on the Superose 6 column. As anticipated, this resulted in a clear shift of SRC-1 immunoreactivity to fractions containing significantly larger

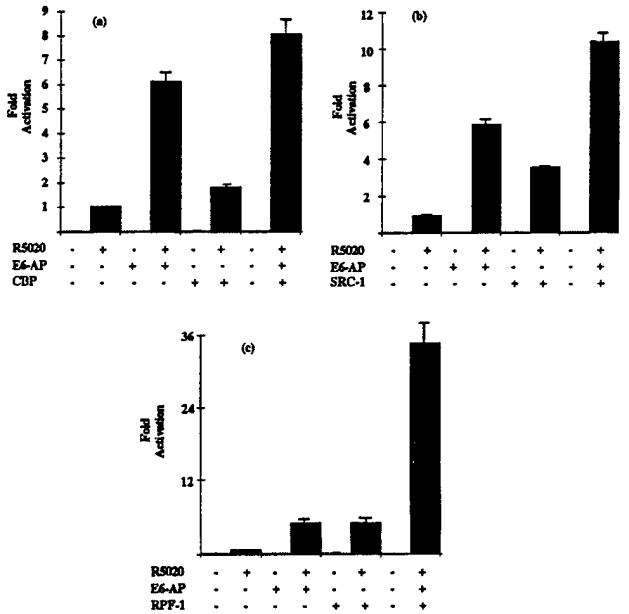

FIG. 3. Synergistic enhancement of PR transactivation by E6-AP and RPF-1. HeLa cells were transiently transfected with 0.2 μg of PR-B expression plasmid and 1 μg of pPRE-E1b-Luc reporter in the presence and absence of 0.5 μg (total) of vectors expressing the indicated coactivators. The cells were treated with either vehicle only (−R5020) or 10nM R5020 (+). Data are expressed as the mean (± SD) of triplicate values.

protein complexes (compare Fig. 4 without anti SRC-1 antibody, fractions 33–35 and Fig. 4 with anti-SRC-1 antibody, fractions 28–32). Stripping and reprobing the same blot with anti-TIF2 antibody indicated a considerable shift of the immunoreactive TIF2 into fractions containing shifted SRC-1 (compare Fig. 4 without SRC-1 antibody, fractions 31–36 and Fig. 4 with anti-SRC-1 antibody, fractions 28–32). To demonstrate that the coeluting of TIF2 and shifted SRC-1 was not due to nonspecific primary or secondary antibody binding, the blot was stripped and reprobed with anti-CBP antibody demonstrating that CBP eluted in the same fractions irrespective of preincubation of lysate with SRC-1 antibody (data not shown). Because the monoclonal SRC-1 antibody does not cross react with TIF2, we take these results to indicate that TIF2 and SRC-1 can form common complexes. As shown earlier, the broader fractionation profile of TIF2 with respect to SRC-1 (Fig. 2) indicates that TIF2 likely also exists in complexes distinct from that which it forms with SRC-1. This is supported by the fact that, although incubation with SRC-1 antibody results in significant shift in the SRC-1 elution profile, a proportion of TIF2 is not shifted by anti-SRC-1 antibody (Fig. 4). Taken together, our results indicate that SRC-1 family members may associate with each other in heteromultimeric protein complexes.

Liganded PR Recruits Preformed Complexes Containing SRC-1 and TIF2 *in Vivo*. To address the relationship of nuclear receptor with these coregulator complexes, we examined their relative migration patterns in the presence and absence of ligand. T47D cells were used for these experiments given their elevated endogenous levels of PR. Lysate from cells pretreated with vehicle or with hormone was subjected to fractionation on the Superose column. Unliganded PR A and B forms eluted in fractions containing protein complexes in the range of ≈500-kDa (Fig. 5*a*, *i*, lanes 32–39, longer exposure of 5*a*, *i*, lanes 32–41), masses consistent with previous reports (1, 37). In the presence of hormone, the liganded PR-B form copurified sharply with the elution peaks of SRC-1 and TIF2 (Fig. 5*a*, *ii*, lane 34; compare with Fig. 2, SRC-1 and TIF2). The liganded PR A form also coeluted with the peaks of SRC-1 and TIF2 but significant amounts did not (Fig. 5*a*, *ii*, lanes 36–41). Liganded PR was largely absent from fractions in which the majority of cellular p300/CBP eluted (compare Fig. 5*a*, *ii* with Fig. 2, p300/CBP).

The presence of the liganded PR forms in fractions containing the peaks of SRC-1 and TIF2 was not conclusive evidence *per se* of an association of PR, SRC-1, and TIF2. To address more precisely the association of liganded PR with the SRC-1 and TIF2-containing complexes *in vivo*, we incubated SRC-1 antibody and polyclonal anti-mouse IgG with T47D lysates prepared from cells pretreated with and without hormone. After fractionation of T47D lysate preincubated with SRC-1 antibody, the elution pattern of the unliganded PR forms was largely unaltered (compare Fig. 5*b*, *i* with Fig. 5*a*, *i*),

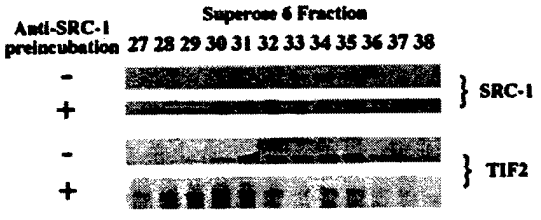

FIG. 4. SRC-1 and TIF2 can form common complexes *in vivo*. SRC-1 complexes were collected by incubation with SRC-1 monoclonal antibody and polyclonal antimouse IgG and fractionated by gel filtration. Immunoblotting confirmed the shift of SRC-1 from its elution peak in the absence of preincubation with anti-SRC-1 antibody (−) to earlier fractions in the presence of anti-SRC-1 antibody (+). The relatively broad elution profile of shifted SRC-1 is most likely due to the heterogeneity of immune complexes formed in these fractions.

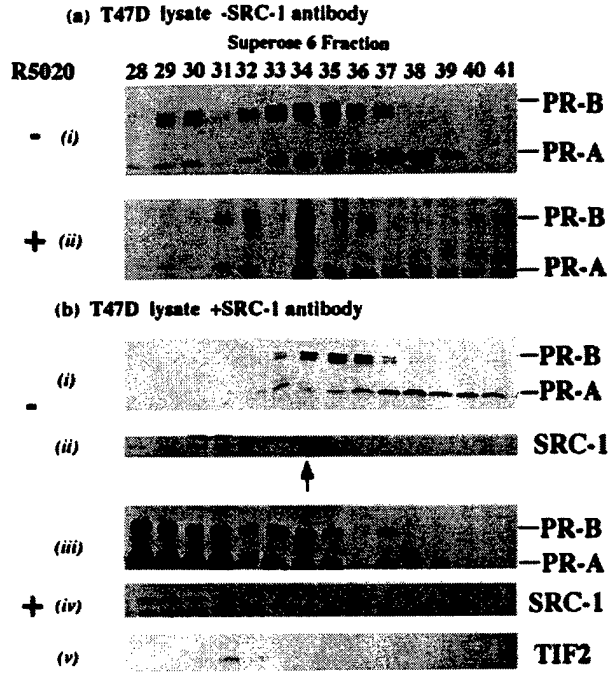

FIG. 5. Liganded PR exists in stable complexes containing SRC-1 and TIF2 *in vivo*. (*a*) T47D cells were pretreated with vehicle (*i*) and with 1nM progesterone (*ii*) before fractionation and immunoblotting with PR antibody. (*b*) Cells were treated as above except lysate was incubated with anti-SRC-1 antibody, fractionated and immunoblotted for (*i*) PR, (*ii*) SRC-1, (*iii*) PR, (*iv*) SRC-1, and (*v*) TIF2. (The arrow indicates the peak of SRC-1 and TIF2 in the absence of preincubation with the SRC-1 antibody).

but SRC-1 was shifted to earlier fractions as predicted (Fig. 5*b*, *ii*, lanes 29–32). In contrast, after ligand treatment of T47D cells, preincubation of lysate with SRC-1 antibody resulted in the shifting of 60–70% of liganded PR A and B forms (Fig. 5*b*, *iii*, lanes 28–31) into fractions containing supershifted SRC-1 (Fig. 5*b*, *iv*, lanes 28–31) and TIF2 (Fig. 5*b*, *v*, lanes 30–31). The relatively broad elution profile of shifted liganded PR (compare Fig. 5*b*, *iii* with Fig. 5*a*, *ii*) is most likely due to the heterogeneity of immune complexes formed in these fractions. A significant proportion of liganded PR A and B forms was not shifted (Fig. 5*b*, *iii*), suggesting that liganded PR also may exist in complexes that do not bind SRC-1 antibody. Our data suggest that, *in vivo*, complexes containing SRC-1 and TIF2 associate stably with PR A and B forms in a ligand-dependent manner.

DISCUSSION

The formation of coregulatorsomes, or multicoregulator complexes, at hormone-regulated promoters has been widely postulated on the basis of multiple interactions between nuclear receptors and coregulators. Inferences as to the nature of the associations within these complexes have been founded largely on functional assays. In particular, the question has been raised of whether these coregulatorsomes associate in the steady-state or whether pools of specific precursor complexes exist. Our data provide direct evidence of the existence *in vivo* of stable subcomplexes of distinct nuclear receptor coregulator subclasses, possibly reflecting established functional differences between these subclasses of coregulators. We suggest that this physical partition of different subclasses of coactivators affords the potential for their efficient combinatorial assembly into higher order complexes. This is consistent with the functional data of Korzus *et al.* (38), which suggest that the requirement for maximal transcriptional activation at specific

promoters may be a function of the existence of diverse groups of coactivator complexes. From our data, it is plausible that transient interactions between the stable subcomplexes we have observed would facilitate rearrangement of final coregulator complexes into multiple configurations.

One issue that is unclear from our data is whether the complexes we have observed represent component parts of larger transcriptional complexes, the kinetics of formation of which do not withstand our experimental conditions. Coimmunoprecipitation and *in vitro* experiments have detected interactions between SRC-1 and other subclasses of nuclear receptor coregulators such as p300 (32), CBP (3), and PCAF (13), as well as interactions between receptor and CBP (3), p300 (32), BRG-1(27), and PCAF (13). Our assay differs from these experiments in that we have been able to analyze multiple coregulator complexes in terms of the relative strengths of their steady–state interactions. In our assay, while SRC-1 was undetectable in fractions containing CBP (Fig. 2), we did observe some overlap of SRC-1 with minor pools of p300 and PCAF (Fig. 2). Interestingly, we also were able to copurify SRC-1 and small amounts of BRG-1 (Fig. 2), raising the possibility that these coregulators form stable steady–state complexes. Our data indicate however that putative complexes between SRC-1/BRG-1, SRC-1/p300, PR/BRG-1, and SRC-1/PCAF, in the steady–state of the cell, represent only small pools of the total amount of these proteins in the cell. In the context of our assay, it is possible that "final" transcriptional complexes are disrupted into the smaller, stable subcomplexes we have observed. However, we have reproduced the elution pattern of previously established complexes under our experimental conditions, such as the mammalian SWI/SNF complex (35, 36). Because we do not observe them under our conditions, final complexes comprised of different subcomplexes may be inherently labile and subject to rapid rearrangement, a plausible mechanism of fine control at transcriptionally active promoters. Additionally, we have not yet detected monomeric forms of coregulators *in vivo*, suggesting that an important mechanism of control of transcription may be the kinetic instability of the monomeric forms of coregulators.

The identification of the stable association of SRC-1 and TIF2 in a single complex, as well as the ability of SRC-1 to homomultimerize, suggests that protein-protein interactions between SRC-1 family members is important for their function *in vivo*. The sequence conservation between family members within the Per-Arnt-Sim/basic helix–loop–helix homology domains, taken together with our data, lends credence to the possibility that the Per-Arnt-Sim/basic helix–loop–helix homology domains mediate this interaction, but this is yet to be established. One consequence of this multimerization might be to increase the number of binding interfaces at which afferent signaling pathways might integrate with promoter-bound receptor.

The precise copurification of the functionally related coactivators E6-AP and RPF-1 in 200–300 kDa complexes is evidence that these proteins may form a stable complex *in vivo*. In light of the cooperative enhancement of PR transactivation by E6-AP and RPF-1, but not E6-AP/SRC-1 and E6-AP/CBP, we speculate that the putative physical association of E6-AP and RPF-1 in common complexes may be related to their synergism. Interestingly, SRC-1 and TIF2, while they can form common complexes, do not synergistically enhance transactivation by PR (data not shown). We suggest this anomaly is due to the fact that E6-AP and RPF-1 have different downstream targets, E6-AP being involved in p53 and HHR23A ubiquitination (39, 40), whereas RPF-1 is required for RNA pol II ubiquitination (41). Conversely, the HAT activities of SRC-1 and TIF2 probably have similar downstream chromatin targets and are likely to be redundant in cotransfection assays. Further studies are required to establish more clearly whether the mechanistic basis of the synergism of E6-AP and RPF-1 is related to their possible existence in a common complex.

Our demonstration of the ligand-dependent association of PR with the SRC-1/TIF2 complex is the first direct evidence that liganded PR associates stably with large coregulator complexes as a distinct step in transactivation *in vivo*. We have shown that unliganded PR forms stable complexes over the range of 400–500 kDa, consistent, within the error of the column, with previous estimates for unliganded PR complexes (1, 37). Liganded PR associates stably with similar sized complex(es) that contain SRC-1 and TIF2. The interaction between activated PR and SRC-1/TIF2 complexes that we have demonstrated is clearly a stable interaction *in vivo*, in comparison to any interaction with CBP or p300. Because liganded PR did not coelute with the major elution peaks of CBP or p300 in the context of our assay, we suggest that activated PR does not recruit these proteins in a stable complex. Rather, our data indicate that liganded PR associates stably with the major peaks of SRC-1 and TIF2, indicating that the complexes within these fractions may represent important fundamental intermediates in PR transactivation. Although our assay is not open to functional interpretation, it is possible that these stable PR/SRC-1/TIF2 complexes undergo relatively transient interactions with other subclasses of coregulators during transcriptional regulation. Our laboratory has suggested (42) that subsequent to formation of a stable committed complex, a "rapid-start" complex is assembled by liganded PR for subsequent rounds of transcription of a template. The relative stability of the liganded PR/SRC-1/TIF2 complexes, makes them plausible candidates for such a rapid-start complex. To further support such a notion, it has been shown that the functional requirement of p300 for estrogen receptor transactivation *in vitro* is reduced before transcriptional reinitiation (43), suggesting that the interaction of p300 with liganded estrogen receptor may be relatively transient. Future work will clarify the functional components

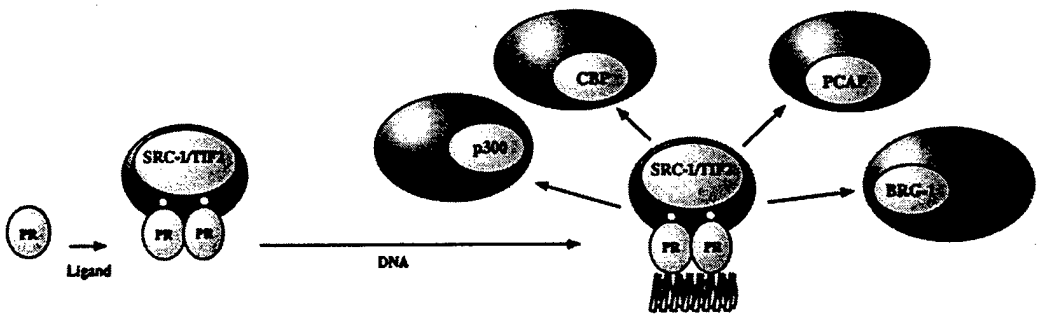

FIG. 6. Mechanistic model for transcriptional activation by activated PR. The relative stability of the complexes between liganded PR and SRC-1/TIF2-containing subcomplexes suggests they may be important intermediates in PR transactivation. Interactions of SRC-1 with other subclasses of coregulators appear to be comparatively transient.

of the complexes with which activated PR associates stably *in vivo*.

Transcriptional regulation by nuclear receptors is increasingly being seen as a modular process, involving multiple discrete steps, such as chromatin remodeling and recruitment of basal transcription factors (27, 43, 44). As a mechanistic basis for this, the multiple distinct subcomplexes we have identified here afford the possibility for their stepwise, sequential interactions with liganded receptor during transcriptional activation. A model based on our data (Fig. 6) suggests that hierarchical interactions, of varying stability, may contribute to transcriptional regulation by PR and coregulators. In our model, liganded PR, SRC-1, and TIF2 are present in comparatively stable core complexes that undergo relatively transient associations with other subcomplexes during transcriptional initiation. In support of such a notion, Fondell *et al.* (45) have identified a class of thyroid receptor-interacting proteins that copurify with constitutively liganded thyroid receptor. These thyroid receptor-interacting proteins are distinct from any coregulator class previously characterized and indicate that liganded receptor may undergo sequential interactions with different multiprotein complexes during transcriptional regulation. Future work will discern the functional significance of these and other complexes and their roles in regulation of gene expression by nuclear receptors.

We thank members of the O'Malley laboratory for helpful discussions and critical reading, M. Burcin and J. Wong for recombinant proteins, D. Edwards for the E6-AP and SRC-1 antibodies, N. Weigel for the PR antibody, D. McDonnell for the RPF-1 antibody and RPF-1 construct, P. Chambon for the TIF2 antibody, R. Eckner for the p300 antibody, W. Wang for the BRG-1 and BAF-57 antibodies, Y. Nakatani for the PCAF antibody, and R. Goodman for the CBP construct. This work was supported by a National Institutes of Health grant.

1. Tsai, M.-J. & O'Malley, B. W. (1994) *Annu. Rev. Biochem.* **63,** 451–486.
2. Onate S. A., Tsai S. Y., Tsai, M.-J. & O'Malley, B. W. (1995) *Science* **270,** 1354–1357.
3. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K., *et al.* (1996) *Cell* **85,** 403–414.
4. Halachmi, S., Marden, E., Martin, G., Mackay, H., Abbondanza, C. & Brown, M. (1994) *Science* **264,** 1455–1458.
5. Hong, H., Kohli, K., Trivedi, A., Johnson, D. L. & Stallcup, M. R. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 4948–4952.
6. Hong, H., Kohli, K., Garabedian, M. J. & Stallcup, M. R. (1997) *Mol. Cell. Biol.* **17,** 2735–2744.
7. Voegel, J. J., Heine, M. J., Zechel, C., Chambon, P. & Gronemeyer, H. (1996) *EMBO J.* **15,** 3667–3675.
8. Torchia. J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K. & Rosenfeld, M. G. (1997) *Nature (London)* **387,** 677–684.
9. Chen, H., Lin, R., Schiltz, L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M., Nakatani, Y. & Evans, R. (1997) *Cell* **90,** 569–580.
10. Li, H., Gomes, P. J. & Chen, J. D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 8479–8484.
11. Anzick, S. L., Kononen, J., Walker, R. L., Azorsa, D. O., Tanner, M. M., Guan, X. Y., Sauter, G., Kallioniemi, O. P., Trent, J. M. & Meltzer, P. S. (1997) *Science* **277,** 965–968.
12. Takeshita, A., Cardona, G. R., Koibuchi, N., Suen, C.-S. & Chin, W. W. (1997) *J. Biol. Chem.* **272,** 27629–27634.
13. Spencer, T., Jenster, G., Burcin, M., Allis, C. D., Zhou, J., Mizzen, C. A., McKenna, N. J., Onate, S. A., Tsai S. Y., Tsai, M.-J. & O'Malley, B. W. (1997) *Nature (London)* **389,** 194–198.
14. Lindebro, M. C., Poellinger, L. & Whitelaw, M. L. (1995) *EMBO J.* **14,** 3528–3539.
15. Chrivia, J. C., Kwok, R. P., Lamb, N., Hagiwara, M., Montminy, M. R. & Goodman, R. H. (1993) *Nature (London)* **365,** 855–859.
16. Eckner, R., Ewen, M., Newsome, D., Gerdes, M., DeCaprio, J. A., Lawrence, J. B. & Livingston, D. M. (1994) *Genes Dev.* **8,** 869–885.
17. Bhattacharya, S., Eckner, R., Grossman, S., Oldread, E., Arany, Z., D'Andrea, A. & Livingston, D. M. (1996) *Nature (London)* **383,** 344–347.
18. Smith, C. L., Onate, S. A., Tsai, M.-J. & O'Malley, B. W. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 8884–8888.
19. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H. & Nakatani, Y. (1996) *Cell* **87,** 953–959.
20. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H. & Nakatani, Y. (1996) *Nature (London)* **382,** 319–324.
21. Jenster, G., Spencer, T. E., Burcin, M. M., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 7879–7884.
22. Blanco, J. C., Minucci, S., Lu, J., Yang, X.-J., Walker, K., Chen, H., Evans, R. M., Nakatani, Y. & Ozato, K. (1998) *Genes Dev.* **12,** 1638–1651.
23. Yoshinaga, S. K., Peterson, C. L., Herskowitz, I. & Yamamoto, K. R. (1992) *Science* **258,** 1598–1604.
24. Muchardt, C. & Yaniv, M. (1993) *EMBO J.* **12,** 4279–4290.
25. Chiba, H., Muramatsu, M., Nomoto, A. & Kato, H. (1994) *Nucleic Acids Res.* **22,** 1815–1820.
26. Ostlund-Farrants, A. K., Blomquist, P., Kwon, H. & Wrange, O. (1997) *Mol. Cell. Biol.* **17,** 895–905.
27. Fryer, C. J. & Archer, T. K. (1998) *Nature (London)* **393,** 88–91.
28. Imhof, M. O. & McDonnell, D. P. (1996) *Mol. Cell. Biol.* **16,** 2594–2605.
29. Cavailles, V., Dauvois, S., L'Horset, F., Lopez, G., Hoare, S., Kushner, P. J. & Parker, M. G. (1995) *EMBO J.* **14,** 3741–3751.
30. Yeh, S. & Chang, C. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5517–5521.
31. Chang, K. H., Chen, Y. M., Chen, T. T., Chou, W. H., Chen, P. L., Ma, Y. Y., Yangfeng, T. L., Leng, X. H., Tsai, M.-J., O'Malley, B. W., *et al.* (1997) *Proc. Natl. Acad. Sci. USA* **94,** 9040–9045.
32. Hanstein, B., Eckner, R., DiRenzo, J., Halachmi, S., Liu, H., Searcy, B., Kurokawa, R. & Brown, M. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 11540–11545.
33. Yao, T. P, Ku, G., Zhou, N., Scully, R. & Livingston, D. M. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 10626–10631.
34. Kee, B. L., Arias, J. & Montminy, M. R. (1996) *J. Biol. Chem.* **271,** 2373–2375.
35. Wang, W., Cote, J., Xue, Y., Zhou, S., Khavari, P. A., Biggar, S. R., Muchardt, C., Kalpana, G. V., Goff, S. P., Yaniv, M., *et al.* (1996) *EMBO J.* **15,** 5370–5382.
36. Wang, W., Xue, Y., Zhou, S., Kuo, A., Cairns, B. R. & Crabtree, G. R. (1996) *Genes Dev.* **10,** 2117–2130.
37. Rehberger, P., Rexin, M. & Gehring, U. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8001–8005.
38. Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T. M., Glass, C. K. & Rosenfeld M. G. (1998) *Science* **279,** 703–707.
39. Huibrgetse, J. M., Scheffner, M. & Howley, P. M. (1993) *Mol. Cell. Biol.* **13,** 4918–4927.
40. Huibregtse, J. M., Scheffner, M. & Howley, P. M. (1993) *Mol. Cell. Biol.* **13,** 775–784.
41. Gavva, N. R., Gavva, R., Ermekova, K., Sudol, M. & Shen, C. K. (1997) *J. Biol. Chem.* **272,** 24105–24108.
42. Klein-Hitpass, L., Tsai, S. Y., Weigel, N. L., Allan, G. F., Riley, D., Rodriguez, R., Schrader W. T., Tsai, M.-J. & O'Malley, B. W. (1990) *Cell* **60,** 247–257.
43. Kraus, W. L. & Kadonaga, J. T. (1998) *Genes Dev.* **12,** 331–342.
44. Wong, J., Shi, Y. B. & Wolffe, A. P. (1997) *EMBO J.* **16,** 3158–3171.
45. Fondell, J. D., Ge, H. & Roeder, R. G. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 8329–8333.

Cell, Vol. 97, 17–27, April 2, 1999, Copyright ©1999 by Cell Press

A Steroid Receptor Coactivator, SRA, Functions as an RNA and Is Present in an SRC-1 Complex

Rainer B. Lanz,* Neil J. McKenna,*
Sergio A. Onate,* Urs Albrecht,
Jiemin Wong,* Sophia Y. Tsai,*
Ming-Jer Tsai,* and Bert W. O'Malley*
*Department of Cell Biology
Department of Biochemistry
Baylor College of Medicine
Houston, Texas 77030

Summary

Nuclear receptors play critical roles in the regulation of eukaryotic gene expression. We report the isolation and functional characterization of a novel transcriptional coactivator, termed steroid receptor RNA activator (SRA). SRA is selective for steroid hormone receptors and mediates transactivation via their amino-terminal activation function. We provide functional and mechanistic evidence that SRA acts as an RNA transcript; transfected SRA, unlike other steroid receptor coregulators, functions in the presence of cycloheximide, and SRA mutants containing multiple translational stop signals retain their ability to activate steroid receptor dependent gene expression. Biochemical fractionation shows that SRA exists in distinct ribonucleoprotein complexes, one of which contains the nuclear receptor coactivator steroid receptor coactivator 1. We suggest that SRA may act to confer functional specificity upon multiprotein complexes recruited by liganded receptors during transcriptional activation.

Introduction

Nuclear receptors are members of a structurally and functionally related family of ligand-activated and sequence-specific eukaryotic transcription factors. By modulating the transcription of target genes in response to their own ligands and other afferent signals, they play key physiological roles in the regulation of development, metabolism, and reproduction. Receptor activation is a multifaceted cascade of events that results in the binding of the receptor to specific regulatory DNA sequences and culminates in the modulation of target gene expression (Tsai and O'Malley, 1994; Mangelsdorf and Evans, 1995). Common to nearly all nuclear receptors is the activation function AF2 in the distal carboxyl terminus of the ligand-binding domain (LBD). A highly conserved amphipathic helix in AF2 has been shown to be important for ligand binding and hormone-dependent transactivation (Danielian et al., 1992; Vegeto et al., 1992; Lanz and Rusconi, 1994). The variable amino-terminal domain of nuclear receptors is extended in the type I or classical receptor subclass comprising the receptors for androgens (AR), estrogens (ER), glucocorticoids (GR), mineralocorticoids (MR), and progestins (PR). This modulatory domain contains a strong and autonomous transactivation function (AF1) that has been shown to be critical for target gene specificity (Tora et al., 1988).

The role of activated nuclear receptors is to direct the assembly and stabilization of a preinitiation complex in a transcriptionally permissive environment at the promoter of a target gene. This involves the functional interaction of the receptor with factors contained in the transcription preinitiation complex (Tsai et al., 1987; Beato and Sanchez-Pacheco, 1996) and with other DNA-bound transcription activators (Jonat et al., 1990). Such interactions are necessary but not sufficient for accurate regulation of transcription. Initial findings that distinct receptors interfere with or squelch each other's transactivation (Meyer et al., 1989) indicated that common limiting factors were involved. Several biochemical and genetic screens have since identified a number of proteins that interact with activated receptors. By fulfilling a number of functional criteria, these coregulators have been defined as coactivators for nuclear receptors (Horwitz et al., 1996; McKenna et al., 1999): they significantly enhance transactivation without altering basal transcriptional activity; when overexpressed, they specifically reverse squelching between different receptors; and they contain autonomous, transferable activation domains.

Coactivators that have recently received considerable attention are members of the SRC gene family and the cointegrators p300 and CBP. Steroid receptor coactivator 1 (SRC-1) was cloned in our laboratory as a general coactivator for nuclear receptors (Onate et al., 1995) and has been termed variously as p160/NCoA-1 (Kamei et al., 1996) or ERAP-160 (Halachmi et al., 1994). Highlighting the critical physiological role of coactivators, the targeted deletion of SRC-1 causes partial hormone insensitivity (Xu et al., 1998). Other nuclear receptor coactivators have been subsequently identified and characterized that are structurally and functionally related to SRC-1 (McKenna et al., 1999 and references therein). The cointegrators CREB-binding protein (CBP; Chrivia et al., 1993) and the closely related adenovirus E1A-associated p300 (Eckner et al., 1994) are well characterized as general coactivators that interact not only with multiple nuclear receptors but with a wide variety of other transcriptional activators.

To date, the majority of receptor-interacting factors have been identified by genetic screens, such as the yeast two-hybrid system, typically using the LBD of a given nuclear receptor as bait. This approach has led to the identification of multiple AF2 coactivators with common structural and functional features. The classical type I steroid receptors, however, exert transactivation via their amino-terminal transcription activation function, AF1. For some steroid receptors, AF1 and AF2 have a distinct pattern of cell and promoter specificity (Bocquel et al., 1989; Tasset et al., 1990). Reasoning that specificity in steroid receptor mediated transactivation might be provided by factors that associate with the poorly conserved AF1, we searched for coregulators

To whom correspondence should be addressed (e-mail: berto@bcm.tmc.edu).

that interact with the amino-terminal AF1 domain of human PR (hPR). We report here the cloning and characterization of a novel transcription coregulator termed steroid receptor RNA activator (SRA). We provide functional and mechanistic evidence that SRA acts as an RNA transcript and exists in a ribonucleoprotein complex that contains the AF2 coactivator SRC-1.

Results

Characterization of *SRA*

In an attempt to find cofactors that interact with steroid hormone receptors, our laboratory used different functional domains of the hPR as baits in a yeast two-hybrid screening system. We previously reported the isolation and characterization of a protein, SRC-1, that interacts with the hPR-LBD (Onate et al., 1995). We performed a similar screen of a human B-lymphocyte library with the AF1-containing amino terminus of hPR_A (corresponding to amino acids 165 567 of hPR_B). Primary sequence analysis of two positive clones from this screen indicated a short open reading frame (ORF). The 3′ extension of reverse-transcribed skeletal muscle poly(A)$^+$ RNA identified an extended ORF with sequence identical to the 5′ ORF from the lymphocyte library.

In order to retrieve cDNAs encoding full-length SRA, we used conventional screening of three different human cDNA libraries from skeletal muscle, heart, and the HeLa S3 cell line. We obtained 13 positive clones with DNA sequences that were identical in a central region. Three variants of SRA were predicted, all containing unique 5′ and 3′ extensions beyond an identical 687 bp core sequence (Figures 1A and 2). We also screened a human genomic DNA library and found two clones with partial sequence identity with the original SRA clones. Additionally, screening of a mouse genomic DNA library identified 5 positive clones, and screening of a mouse cDNA library found 14 positive clones, of which 2 revealed 75% identity to the human SRA cDNA (Figure 2). Primary sequence analysis of clones from different human and mouse cDNA and genomic DNA libraries suggested that SRA represented a family of clones highly homologous in a core sequence but divergent in their 5′ and 3′ regions. Sequence comparison using the BLAST algorithm indicated no homologs but identified partial SRA sequences isolated as HepG2-3′UTR (accession number D16861), expressed sequence tag clones, and chromosome 5 BAC clone 319C17 (AC005214), although no functions for these sequences were described.

To determine the expression patterns of the corresponding RNA, we performed Northern analysis using a cDNA probe corresponding to the core sequence of human SRA. Major transcripts of 0.7 0.85 kilobases (kb) in length and less abundant transcripts of 1.3 1.5 kb were detected in a human multiple tissue Northern blot (Clontech) (Figure 1B), indicating that the isolated cDNAs were likely to be full length. In addition, SRA was expressed at different levels in the tissues examined; transcripts were enriched in liver and skeletal muscle but expressed at a low level in brain. Interestingly, the expression of the two messages in the 0.7 0.85 kb doublet appeared to be tissue specific in the multiple tissue blot. A cell line specific expression of these isoforms (represented by the doublet) was also observed in a

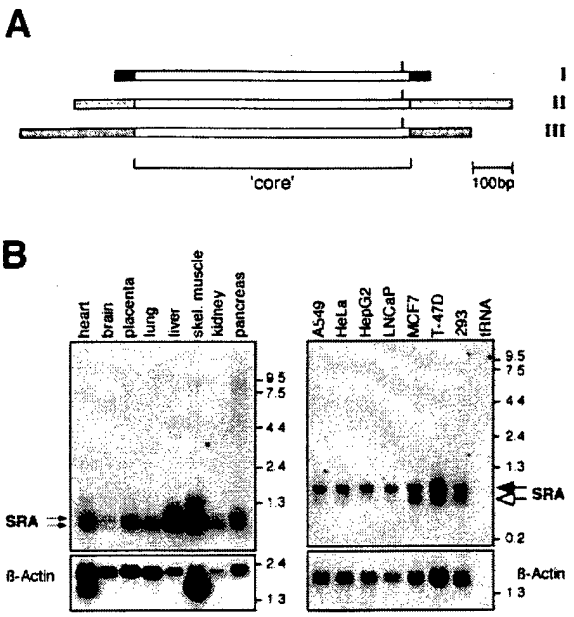

Figure 1. Characterization of *SRA* Genes

(A) Structure of three SRA isoforms (I III) deduced from screening of different cDNA and genomic DNA libraries from human and mouse. The sequences are identical in a core region of 687 bp (no shadow) but are divergent in their 5′ and 3′ sequences (distinct shadings). The vertical lines indicate the location of the proposed termination codon of the putative open reading frame ORF1.

(B) Northern analysis of human *SRA* gene expression. (Left panel) Multiple tissue Northern blot, containing 2 μg of human poly(A)$^+$ RNA from each of the tissues indicated at the top, was hybridized with a cDNA probe corresponding to the core sequence of human *SRA* (A). Predominant transcripts of about 0.7 0.85 kb (double arrows) and less abundant transcripts of 1.3 1.5 kb are apparent. The blot was stripped and reprobed with β-actin to correct for RNA loading (bottom). (Right panel) Northern analysis of human tissue culture RNA probed with the longest cDNA sequence of SRA ([A], isoform III) indicates a cell line specific expression of SRA isoforms. MCF-7 and T-47D cells have significantly higher levels of the smaller SRA transcript (open arrow) compared to other tissues, but they express similar levels of the larger transcript (filled arrowhead). Total RNA was isolated from human cell lines as indicated and 15 μg analyzed. The membrane was subsequently hybridized with β-actin probe as an internal control for loading (bottom). Size markers are indicated on the right (kb).

A549, lung carcinoma; HeLa, epitheloid cervix; HepG2, hepatoblastoma; LNCaP, metastatic prostate adenocarcinoma; MCF-7, breast adenocarcinoma; T-47D, breast ductal carcinoma; 293, transformed primary embryonal kidney.

Northern analysis of poly(A)$^+$-selected mRNA from different human tissue culture cell lines. All of the cell lines tested expressed the ~0.85 kb doublet species, whereas the smaller ~0.7 kb species was expressed at significantly higher levels in the breast cancer cell lines MCF7 and T-47D, compared to the other cell lines investigated (Figure 1B). This isoform-specific expression was conserved in mouse tissue (not shown). We concluded that multiple SRA isoforms are expressed quantitatively in a tissue- and cell type specific manner.

SRA Is a Steroid Receptor Specific Coactivator

To investigate the functional role of SRA, we subcloned the cDNAs into mammalian expression vectors and assayed the effect of SRA on PR-dependent transactivation. HeLa cells were cotransfected with the CMV-hPR

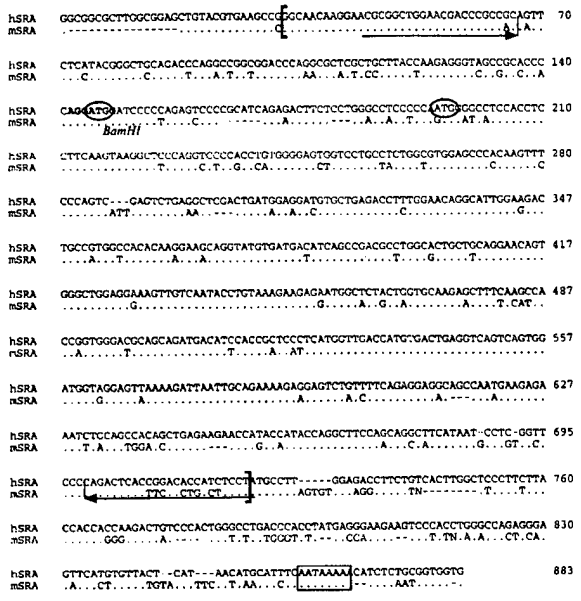

Figure 2. Primary Nucleotide Sequence Alignment of SRA Isoform I cDNA from Human and Mouse

The nucleotides of mouse cDNA are indicated where they differ from the human cDNA sequence. Brackets represent the boundaries of the SRA core sequence. Arrows illustrate the location and orientation of the primer set used for SRA-specific RT-PCR (shown in Figures 6 and 7). The Kozak consensus sequence is marked in bold; circles indicate the putative translation initiation codons (ATG) targeted for mutation analysis (Figure 4); a consensus polyadenylation signal (AATAAAA) is boxed.

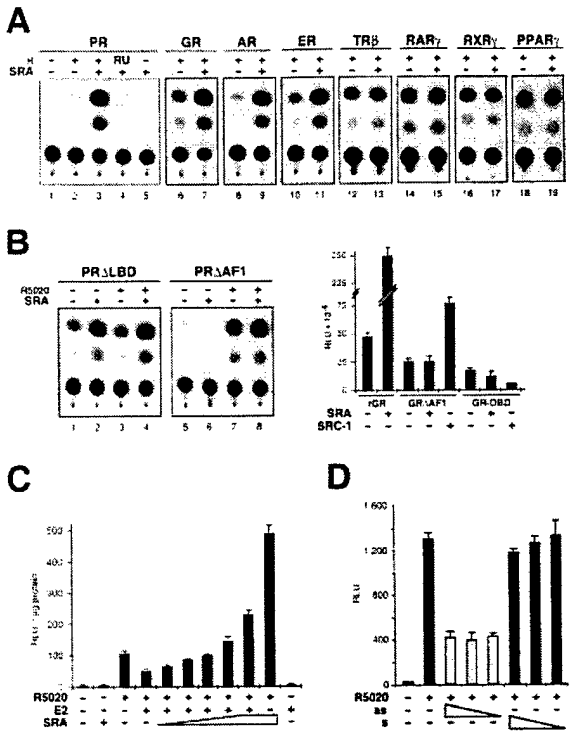

Figure 3. SRA Is a Steroid Hormone Receptor Specific Coactivator

(A) SRA enhances transcription mediated by steroid receptors. Thin-layer chromatographs showing the chloramphenicol-acetyltransferase (CAT) reporter activity as a response to steroid receptor mediated transactivation in the presence of SRA (+) or empty vector (−). HeLa cells were transiently transfected with plasmids encoding the human receptors for glucocorticoid (GR), androgen (AR), estrogen (ER), thyroid hormone (TRβ), all-*trans* retinoic acid (RARγ), 9-*cis* retinoic acid (RXRγ), or with peroxisome proliferator activated receptor (PPARγ) along with their cognate hormone response element coupled to a TATA-CAT reporter gene and induced with their appropriate ligands or the PR antagonist RU486.

(B) SRA enhances transcription via the N-terminal activation domain AF1 of steroid receptors. (Left panel) PR deletion mutants lacking either the ligand-binding domain (PRΔLBD: lanes 1 4) or the amino-terminal activation function (PRΔAF1: lanes 5 8) were assayed as in (A). Similar experiments with rat GR or domains thereof excluded the DNA-binding domain (DBD) as a possible target for SRA (right panel). The GR truncations were fused to the activation domain of GAL4 to monitor transactivation. SRC-1 was used as control. MMTV-driven luciferase gene expression is shown as relative light units (RLU).

(C) Overexpression of SRA reverses squelching of PR by ER in a dose-dependent manner. HeLa cells were transfected with PR and ER expression plasmids (50 ng), MMTV-luciferase reporter (2.5 μg), and different amounts of SRA (0 4 μg) and supplemented with 50 nM receptor-specific ligands R5020 or E2, or both, as indicated. Luciferase activities are shown as RLU per microgram of protein assayed as the mean (±SD) of triplicate values.

(D) PR transactivation is inhibited by digestion of endogenous SRA in an oligonucleotide-dependent manner. HeLa cells were transfected with PR expression plasmid (20 ng) and MMTV-luciferase reporter (2.5 μg) together with different concentrations (200, 100, and 50 nM) of SRA-specific, 2'methoxyethyl-modified antisense deoxyoligonucleotide (as), or sense control (s). Luciferase activities are shown as the mean (±SD) of triplicate values.

and CMV-SRA (CMV, cytomegalovirus) along with $(PRE)_2$-TATA-CAT reporter (CAT, chloramphenicol acetyltransferase) and induced with progestin (R5020). We observed that SRA enhanced PR transactivation (Figure 3A; compare lanes 2 and 3) and that SRA did not alter the activity of PR in the presence of its antagonist RU486 (lane 4). Furthermore, SRA did not significantly elevate the basal activity of the minimal promoter (lane 5). Similar transfection experiments with the human receptors for GR, AR, ER, thyroid hormone (TR), retinoic acid (RAR and RXR) or peroxisome proliferator activated receptor (PPAR), and CAT reporters containing cognate hormone response elements revealed that SRA selectively enhanced steroid receptor mediated transactivation (Figure 3A). SRA did not enhance transactivation induced by other activators such as GAL4, Sp1, E2F, E47, and forskolin-stimulated CREB (data not shown).

We next found that SRA enhanced transactivation through the N-terminal AF1 portion of steroid receptors. Truncation of the A/B domain of the PR (PRΔAF1) significantly reduced coactivation by SRA (Figure 3B, lanes 7 and 8), whereas transcription activation by PR lacking the LBD (PRΔLBD) was fully responsive to SRA (lanes 1 4). In order to exclude the DNA-binding domain as a mediator for SRA coactivation, we tested different domains of rat GR as fusion proteins with the activation domain of GAL4 (Figure 3B, right panel). As expected, neither the amino-terminally truncated GRΔAF1 nor the DNA-binding domain of rat GR (GR-DBD) responded to SRA to enhance luciferase reporter activity. As a control, GRΔAF1 enhanced reporter activity in the presence of SRC-1. These results indicated that SRA mediates transcriptional activation of steroid receptors by a mechanism involving the AF1 of the receptors. We next tested the possibility that intramolecular crosstalk between

the AF1 and AF2 of the receptor is mediated by SRA and SRC-1 but found that coexpression of both coactivators had only an additive effect on the coactivation of PR-mediated transactivation (not shown). To better determine the coactivation potential of SRA in vivo, we transfected different SRA clones into T-47D cells and tested the ability of SRA to enhance the activity of endogenous PR. All three isoforms of SRA cDNA, in addition to a portion of the human genomic SRA, enhanced transactivation mediated by the endogenous PR by 8- to 12-fold, and the core domain of SRA was found to be necessary and sufficient for this coactivation (not shown).

Another criterion for classification as a coactivator is the ability of a factor to reverse interference (squelching) by transcriptional activators with common coregulators. To ask if SRA is a limiting factor that can be sequestered by an excess of another receptor, we used a PR-regulated gene reporter assay in the presence of ER (Figure 3C): while ligand-activated ER reduced the transcription activity of PR by 50%, full PR transactivation was reestablished by addition of SRA, confirming that SRA regulates the transactivation of both PR and ER in a dose-dependent manner and indicating that SRA is a limiting cellular factor for steroid receptors.

In order to assess the effect of abrogation of endogenous SRA on PR-mediated transcription, we developed an assay in which SRA transcripts were selectively degraded. By administration of stabilized antisense deoxyoligonucleotides, we attempted to digest SRA by endogenous RNase H, an endoribonuclease that specifically hydrolyzes the phosphodiester bonds of RNA/DNA hybrids. Figure 3D shows that PR target gene expression was reduced by ~70% by cotransfection of an SRA antisense 2'methoxyethyl oligonucleotide, whereas the corresponding sense construct had no effect on PR transactivation. Similar experiments were performed with three other SRA-specific deoxyoligonucleotides, all of which significantly reduced PR target gene expression. In addition, the antisense oligonucleotides were capable of reducing reporter gene expression mediated by endogenous receptor (not shown). These results imply that endogenous SRA has a direct impact on steroid-mediated transcription in vivo. Together with the ability of SRA to enhance transactivation on minimal and natural promoters without altering basal transcription, these results clearly characterized SRA as a bona fide coactivator, specific for the AF1 domain of steroid receptors.

SRA Does Not Exhibit Characteristics of a Protein

Sequence analysis of the SRA clones indicated an ORF terminated at the 3' end of the core sequence (see Figure 1A; a detailed ORF map is shown in Figure 4A: this ORF is denoted ORF1). A second ORF, ORF2, contains a consensus Kozak sequence in the 5' portion of the SRA cDNA. This ORF2 corresponds to the presumed receptor-interacting reading frame of the yeast hybrid clones. However, an in-frame stop codon terminated GAL/SRA fusion products prematurely at the 5' end of the core sequence. We concluded that the interaction of the original yeast two-hybrid SRA clones with the AF1 of PR was unlikely to have been mediated by a protein product encoded by ORF2 of SRA.

We next attempted to characterize the presumptive

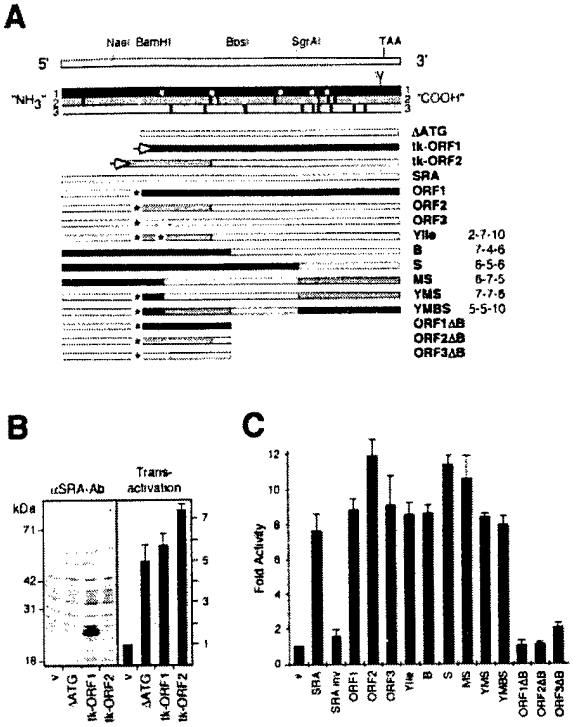

Figure 4. Mutated SRA Constructs Enhance PR Transactivation

(A) Schematic presentation of SRA mutants. Top: SRA core cDNA and deduced ORF map; selected restriction sites and presumptive termination codon for ORF1 (TAA) are indicated; Y, location of peptide sequence used to generate SRA-mAb; vertical lines in ORF map, stop codons; white, putative initiation codons. Bottom: SRA mutants used in the transactivation studies presented and described in (B) and (C). Triple numbers indicate total of stop codons in each ORF; asterisks, point mutation(s); arrows, translation initiation region of the thymidine kinase promoter (tk); the shade of gray indicates reading frames of presumptive translation products: white, unconstrained; black, ORF1 (recognized by mAb); gray, ORF2; light gray, ORF3.

(B) Immunodetection and coactivation of transfected SRA mutants indicate that SRA coactivates PR transactivation in an ORF-independent manner. SRA mutants along with MMTV-Luc reporter and PR expression plasmid were transfected into COS cells and analyzed for immunoreactivity to SRA antibody raised against a peptide sequence deduced from the C terminus of ORF1 (left panel) and for coactivation (right panel). Mutants are as follows: (1) N-terminal truncation at the intrinsic Kozak sequence (ΔATG) and (2) fusion with translation initiation region of the tk in two different open reading frames (tk-ORF1 and tk-ORF2). The constructs are illustrated in (A). Protein size markers are indicated on the left. Fold coactivation in relation to PR transcription as the mean (±SD) of triplicate values is indicated on the right.

(C) Enhancement of PR-mediated transactivation of the MMTV-Luc reporter by various mutated SRA constructs shown schematically in (A). Fold coactivation is indicated relative to expression of empty vector (v) and shown as the mean (±SD) of triplicate values.

SRA, wild-type SRA; SRA inv, cDNA of SRA expressed in 3' 5' orientation; ORF1, ORF2, ORF3, nonsense mutations at the BamHI site obliterating the Kozak sequence of two reading frames and permitting only one putative translation product; YIle, mutant ORF2 with an additional point mutation altering an ATG (Figure 2) and generating an MfeI site; B, frameshift mutation at the BbsI site of SRA; S, frameshift mutation at the SgrAI site; MS, frameshift mutations at the MfeI and SgrAI sites; YMS, mutant MS with additional frameshift mutation at the BamHI site; YMBS, mutant ORF2 with additional frameshift mutations at MfeI, BbsI, and SgrAI; ORF1ΔB/ORF2ΔB/ORF3ΔB, 3' deletion at BbsI of the mutants ORF1, ORF2, and ORF3, respectively.

SRA protein product. Surprisingly, all our efforts to generate SRA-encoded protein were unsuccessful. In vitro translation of different SRA cDNAs did not result in detectable levels of protein, whereas carboxy-terminal fusions of SRA with GAL4 or GST produced the expected translation products (not shown). In addition, GAL/SRA fusion constructs failed to activate the UAS heterologous promoter, indicating that SRA did not possess an intrinsic activation function. We then generated a monoclonal antibody (mAb) against the peptide sequence encoded by the 3′ end of the SRA core (ORF1). In Western analysis, only ORF1 fusion constructs of GAL/SRA and GST/SRA were immunoreactive (not shown), whereas no proteins generated by expressed SRA cDNAs were detectable in cell extracts. We concluded that the SRA cDNA sequence did not encode a viable translation product.

We then correlated the coactivation function of SRA with its expression. Mutated SRA constructs were transfected into cultured cells and analyzed in a side-by-side comparison for SRA immunoreactivity and for coactivation of PR-mediated transactivation. The constructs tested were (1) a 5′ truncation at the BamHI site (Figure 4A), eradicating the consensus Kozak sequence, and (2) a fusion of this truncated cDNA to the HSV-thymidine kinase initiation sequence (tk) in two distinct reading frames, producing tk-ORF1 and tk-ORF2. All three SRA mutants enhanced PR-mediated transactivation (Figure 4B, right panel), whereas only one construct–the reading frame of which corresponded to ORF1–was recognized by the mAb (4B, left panel). No endogenous SRA protein was detected that corresponded to the constrained translation of tk-ORF1. Importantly, screening of a panel of tissue culture cell lines by matrix-bound SRA-mAb confirmed the absence of endogenous SRA protein in these lines (not shown). Taken together, these results suggested to us that coactivation by SRA was unlikely to be mediated by its presumptive protein product.

In order to substantiate these results, we generated various SRA mutants and tested them in cell culture for their ability to coactivate PR-dependent transcription. Figure 4A shows the sequence of the SRA mutants relative to the original SRA clone. Several of the mutants lacked the ATGs in ORF1 and ORF2; others contained mutations within the Kozak sequence, allowing a presumptive translation of only one given reading frame (see legend to Figure 4A for details). Other constructs contained single or multiple frameshift mutations along the core sequence, resulting in a mosaic organization of reading frames, each containing approximately six translational stop codons on average. A representative assay of in vivo expressed SRA mutants (Figure 4C) clearly demonstrates that most of the SRA mutants retained the ability to enhance PR transcription by 8- to 12-fold. Only SRA expressed in 3′ 5′ orientation (SRA inv) or 3′ half-truncated versions of the ORF exclusion mutations were inactive. Similar results were obtained with mutants of other SRA isoforms (not shown). These results further suggested to us that the coactivation exerted by SRA on steroid receptor transcription was unlikely to be mediated by a translation product of the *SRA* gene.

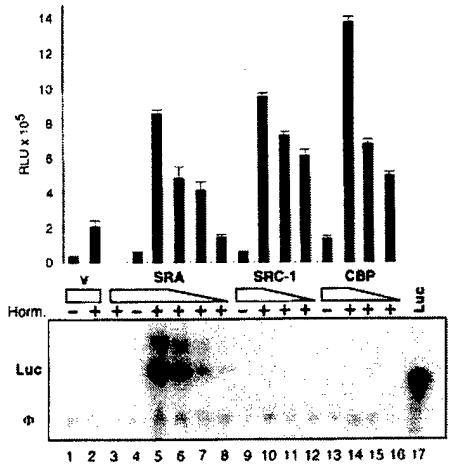

Figure 5. SRA Is an RNA Coactivator

SRA does not require protein synthesis to enhance endogenous GR-mediated transactivation. Two separate groups of HeLa cells were transiently transfected with reporter MMTV-Luc (2 μg) along with different amounts of CMV-driven expression plasmids for SRA (3, 2, 1, 0.5 μg), SRC-1 and CBP (3, 2, 1 μg), or empty vector (v; 3 μg) and treated with EtOH control (−) or dexamethasone (+). One set of transfected cells was assayed for luciferase protein expression (upper panel), the other set of cells incubated in medium containing 50 μM cycloheximide and subjected to RNA isolation and DNaseI digestion, followed by Northern analysis for luciferase RNA expression (lower panel). The Northern blot was hybridized with probes specific for cyclophilin (Φ) and luciferase RNA (Luc). Lane numbers (bottom) are common to both assays. Dilution (10,000-fold) of RNA from cotransfected CMV-luciferase plasmid expression is shown as control in the Northern analysis (lane 17). A longer exposure of the blot revealed low levels of luciferase transcripts in all samples that were treated with dexamethasone.

SRA Is an RNA Coactivator

We next focused our attention on the transcription products of the cDNA encoding SRA. We designed an assay for RNA-mediated transactivation by targeting endogenous GR in HeLa cells cultured in the presence of the de novo protein synthesis inhibitor cycloheximide and asked if SRA retained the ability to coactivate GR-mediated transcription. As controls, we used the coregulators SRC-1 and CBP, both of which interact with nuclear receptors as proteins. Two separate pools of HeLa cells were transiently transfected with an identical mixture of MMTV-luciferase reporter (MMTV, mouse mammary tumor virus LTR) along with CMV-driven expression plasmids for SRA, SRC-1, CBP, or empty vector and treated with carrier or dexamethasone. One set of transfected cells was subjected to a conventional luciferase protein assay for GR-mediated transactivation. The second set of cells was first incubated in medium containing cycloheximide from 3 hr prior to transfection until harvesting and then subjected to Northern analysis for luciferase RNA expression. Figure 5 shows a representative side-by-side comparison of luciferase expression as protein (upper half) and RNA (lower half). As expected, we observed a hormone- and dose-dependent enhancement of transactivation by all coregulators in the absence of cycloheximide (upper panel). The relatively low coactivity for all coactivators resulted from lower protein expression levels due to the necessarily shorter incubation time for cycloheximide-treated cells. In contrast,

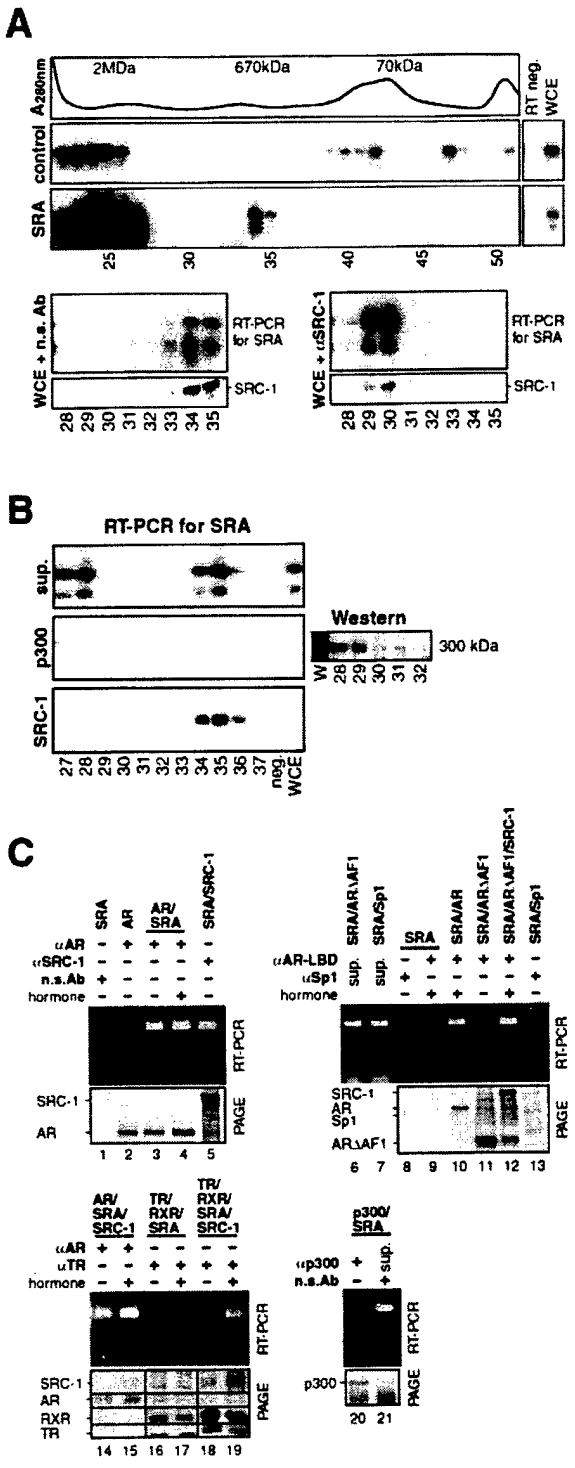

Figure 6. SRA Is Present in an SRC-1 Complex

(A) Copurification of SRA and SRC-1 complexes by gel filtration chromatography. Upper panels: T-47D lysates (~400 μg) were fractionated on a Superose 6 column and analyzed for total protein elution ($A_{280\ nm}$, top), SRC-1-specific RT-PCR (control), and SRA-specific RT-PCR (SRA). RT-PCR analysis of ~20 μg input whole-cell extract in the presence (WCE) or absence of reverse transcriptase (RT neg.) are shown to the right. Numbers indicate fractions. Elution peaks of molecular size markers are given for mammalian SWI/SNF complex (~2 MDa) and thyroglobulin (670 kDa); the void volume (4 MDa for globular proteins) was determined at fraction 20 by silver staining (not shown). Lower panels: SRA-specific RT-PCR and parallel immunoblots with SRC-1-specific antibody of fractionated T-47D cells after preincubation of the lysate with either nonspecific antibody (WCE + n.s. Ab) or SRC-1 antibody (WCE + SRC-1 Ab). In addition to SRA, fractions 34 and 35 contained the AF2 coactivators TIF2 and SRC-3 (McKenna et al., 1998 and not shown). Since SRC-3 was reported to be an exclusively nuclear protein (Suen et al., 1998), it indicates that our lysis conditions extract complexes both of nuclear and cytoplasmic origin.

(B) Coimmunoprecipitations of SRA in fractionated cells. T-47D lysates were fractionated as in (A), subsequently immunoprecipitated with antibodies against p300 (middle) and SRC-1 (bottom), and analyzed for SRA by RT-PCR (left panels) or by parallel Western analysis for precipitation of p300 (right). Numbers indicate fractions. sup., combined supernatant of both precipitation reactions; neg., RT-PCR omitting reverse transcriptase; WCE, input lysate (note that the conditions for immunoprecipitation of fractionated extracts did not coprecipitate SRA in WCE).

(C) Coimmunoprecipitation of SRA with AR or SRC-1 in *Xenopus* oocyte extracts. Ethidium bromide stained agarose gel of SRA-specific RT-PCR products from immunoprecipitation reactions (RT-PCR) and parallel SDS-PAGE analysis of precipitated proteins (PAGE). In vitro transcribed RNAs for SRA, SRC-1, p300, AR and the AR mutant ARΔAF1, TR, RXR, and Sp1 were injected along with L-^{35}S-methionine into *Xenopus laevis* oocytes (as indicated at the top) and the translation products subsequently targeted for immunoprecipitation (antibodies indicated at the left). n.s. Ab, nonspecific antibody; αAR-LBD, polyclonal antibody against the C terminus of androgen receptor (AR); ARΔAF1, AF1-depleted AR; sup., supernatant of immunoprecipitation reaction.

the Northern analysis for luciferase expression of the set of cycloheximide-treated cells (lower panel) revealed that SRA (lanes 5 8), but not SRC-1 (10 12) or CBP (14 16), was able to enhance transcription under these conditions. As a control, we assayed ^{35}S-methionine incorporation in the two groups of cells and found that the amount of cycloheximide used in our assays (50 μM) abolished >99% of the total cellular translation products (not shown). Moreover, a third control set of transfected cells treated with cycloheximide and analyzed for luciferase reporter activity produced relative light units corresponding to basal activity. The fact that only SRA and not SRC-1 and CBP was capable of potentiating GR-mediated transcription in the absence of de novo protein synthesis was clear evidence that the functionality of SRA was not contingent upon translation of the primary SRA transcript.

SRA Is Present in a Distinct Steroid Receptor Coregulator Complex

Given that functional RNAs are known to associate with proteins as ribonucleoprotein complexes, we next asked if SRA might function as a component of similar complexes. To investigate protein SRA interaction in a steady-state situation in vivo, we fractionated whole-cell extract from human T-47D cells on a Superose 6 column as previously described (McKenna et al., 1998). One-half of the collected fractions were processed for Western analysis using specific antibodies against transcriptional coregulators, and the remainder of each fraction was subjected to RNA isolation followed by SRA transcript specific RT-PCR and Southern analysis. We validated our RT-PCR method by incubation of cell extract with SRA-specific antisense deoxyoligonucleotides and subsequent digestion with endoribonuclease

RNase H, which destroyed the SRA signal in an oligonucleotide- and dose-dependent manner (not shown). We found that endogenous SRA specifically eluted in complexes of 600 700 kDa (Figure 6A, fractions 34 and 35). We verified that these fractions do not reflect a nonspecific peak of proteins and RNA (Figure 6A, upper panels). Western analysis indicated that SRA copurified with fractions containing SRC-1 (Figure 6A, SRC-1). Interestingly, SRA was not detected in fractions containing p300/CBP (fractions 28 31). The colocalization of SRC-1 and SRA led us to consider that they may be part of a common complex in vivo. Based on this assumption, we attempted to alter the elution pattern of this putative complex by incubating cell lysates with anti-SRC-1 antibody and rabbit anti-mouse IgG prior to fractionation. As predicted, this resulted in a clear shift of both the SRA signal and SRC-1 immunoreactivity from fractions 34 35 to fractions 29 30 (Figure 6A, lower panel). To exclude the possibility that the shifted SRC-1 was due to nonspecific antibody binding, the Western blots were stripped and reprobed with anti-CBP antibody. The elution profile of CBP was the same irrespective of preincubation of cell lysate with SRC-1 antibody (not shown).

To verify our findings that SRA associates–directly or indirectly–with SRC-1, we attempted to coimmunoprecipitate SRA and SRC-1. To quantitatively enrich SRA in the cells subjected to fractionation, we transfected HeLa cells with a plasmid encoding SRA. We then fractionated the lysates and subjected the fractions to immunoprecipitation with antibodies against SRC-1 and p300 prior to RT-PCR analysis. SRA was detected in the anticipated fractions in both the inputs and the SRC-1 precipitates (Figure 6B, upper and lower panels, fractions 34 36), but SRA was not coimmunoprecipitated with p300 (middle panel). Parallel Western analysis indicated that p300 was specifically precipitated by anti-p300 antibody in the anticipated fractions. These results verified our finding that SRA resides in a complex containing SRC-1 but not p300 or CBP. To test the possibility that SRA might have a structural role in the SRC-1 complex, we treated cell extracts with RNase prior to fractionation. These extracts did not produce SRA signals in the RT-PCR analysis, whereas SRC-1 was detectable in fractions 35 36 (not shown), suggesting that SRA does not have a vital structural role in SRC-1 complexes. Taken together, biochemical fractionation experiments indicated that SRA is a component of distinct ribonucleoprotein complexes, one of which contains the nuclear receptor coactivator SRC-1.

Having established that SRA was present in SRC-1-containing complexes, we wished to know whether SRA interacted with steroid receptors as a component of a ribonucleoprotein complex. To address this possibility, we performed coimmunoprecipitation experiments using a previously described expression system in *Xenopus* oocytes (Wong et al., 1995). In vitro generated RNAs encoding SRA, SRC-1, p300, AR and the AR mutant ARΔAF1, TR, RXR, and the nonnuclear receptor transcription factor Sp1, along with L-^{35}S-methionine, were injected into oocytes and their cell extracts subjected to coimmunoprecipitation with antibodies against the expected protein products. Figure 6C shows the cDNA products generated by SRA-specific RT-PCR of the various immunoprecipitates along with SDS-PAGE analysis. SRA was undetectable after immunoprecipitation using a nonspecific antibody from cell lysates programmed with SRA (lane 1). Similarly, SRA was not detected after immunoprecipitation with an AR antibody from cell lysates injected with RNA encoding AR, although AR was specifically precipitated (lane 2). In contrast, the AR antibody precipitated SRA in a hormone-independent manner in extracts from oocytes injected with RNAs for SRA and AR (lanes 3 and 4). We next investigated the specificity of the interaction of SRA with SRC-1 and AR. An AR mutant lacking the amino-terminal domain (ARΔAF1) did not retain coinjected SRA (lane 11), although the supernatant clearly contained SRA (lane 6). However, in oocytes containing SRC-1 in addition to SRA and ARΔAF1, the antibody against the LBD of AR coprecipitated SRA and SRC-1 (lane 12). In addition, immunoprecipitation using a monoclonal antibody against SRC-1 from oocytes programmed with RNAs for SRA and SRC-1 clearly coprecipitated SRA and SRC-1 (lane 5), verifying that SRA is in a stable association with SRC-1. Similarly, analysis of oocytes programmed with SRA, TR, RXR, and SRC-1 and precipitated with TR antibody indicated SRA only in SRC-1-containing extracts (lane 19), verifying again the selectivity of SRA for steroid receptors and SRC-1. Taken together, we conclude that SRA exists in a ribonucleoprotein complex containing SRC-1 and that this complex is recruited by a steroid receptor.

Discussion

In this work we describe the isolation and functional characterization of a novel transcriptional coactivator termed SRA. SRA is different from other known coregulators in that it functions as an endogenous RNA transcript. We have defined several different features of this RNA: SRA is (1) a bona fide transcriptional coactivator, (2) selective for the AF1 of steroid receptors, (3) expressed as multiple isoforms in a cell-specific manner, and (4) present in a steady-state coregulator complex with the AF2 coactivator SRC-1.

We have described the isolation of three SRA isoforms deduced from sequencing of different cDNAs and genomic clones from different species. When overexpressed in mammalian cells, recombinant SRA, regardless of isoform or origin, enhanced steroid receptor mediated transactivation without significantly enhancing the level of basal transcription of minimal or natural promoters. In assays of endogenous PR mediated transactivation, a typical enhancement of receptor gene activity of ~10-fold was achieved by coexpression of SRA. Antisense deoxyoligonucleotides added to cells reduced steroid receptor induced transcription by up to 70%. In addition, we have shown that SRA reverses steroid receptor squelching in a dose-dependent manner. Hence, SRA exhibits many characteristics expected of a bona fide coactivator.

Despite certain functional similarities, SRA differs in some important aspects from many other coactivators in that its coactivation is selective and that it is an RNA. We have presented several independent lines of evidence that indicate that SRA selectively enhances steroid receptor mediated transactivation but does not influence transactivation by type II nuclear receptors or

by other transcription factors. In addition, using in situ hybridization analysis we have obtained evidence for both a selective expression pattern of SRA and a general colocalization in brain tissue with members of the steroid receptor family (data not shown). In our coimmunoprecipitation assays it appears that, unless SRC-1 is coexpressed, the N-terminal domain of steroid receptors is required for binding of SRA. In our reporter gene assay, SRA per se fails to enhance transcriptional potency of the AF2 receptor domain in cultured cells. The exact nature of the interaction of SRA with the AF1 domain of steroid receptors is as yet unclear. SRA was originally isolated in the yeast two-hybrid system, an assay designed to identify protein protein interactions. In a reconstructed yeast system, SRA associated with the N-terminal domain of PR but not with a control hybrid (not shown). The lack of sequence homology within the amino terminus of steroid receptors suggests that SRA may interact indirectly with the AF1 of the receptors as part of a ribonucleoprotein complex. It is unlikely that protein protein interactions between the bait construct and the GAL activation domain played any role in the isolation of SRA. Rather, we envision an interaction of SRA with the PR N-terminal bait, thereby recruiting it to the reporter gene site. The SRA-PR N terminus interaction is likely to have been supported by yeast proteins, possibly through a mediator with functional similarity to SRC-1, and such interactions would have favored reporter gene activity and resulted in a positive hit. This somewhat fortuitous isolation of SRA appears less puzzling when it is considered that yeast proteins contribute functionally to transcriptional activation by steroid receptors (Yoshinaga et al., 1992), even though steroid receptors are not expressed in yeast.

Although we do not totally exclude the existence of a translation product of SRA contained in certain cells at specific developmental stages, we have provided evidence to indicate that SRA exists and functions as an RNA transcript. First, we were not successful in our attempts to translate the SRA clones in vitro or in vivo. Second, an affinity column containing a mAb raised against a sequence at the carboxy-terminal end of the putative ORF1 transcript failed to detect endogenous SRA in various cell lines tested. In addition, extensive mutagenesis of SRA, introducing multiple translational stop codons in all reading frames, did not affect the ability of these mutants to enhance PR transactivation. A final functional test was provided by transfection experiments in the presence of cycloheximide, in which SRA retained its ability to coactivate a reporter gene, while other protein coregulators such as SRC-1 and CBP did not.

The ability of RNA molecules to perform many functions that were commonly attributed to proteins has been well documented. RNA molecules perform enzymatic reactions such as *trans*-esterification (Jaeger, 1997) or catalysis of peptide bond formation (Zhang and Cech, 1997) and can regulate gene expression in *trans* by structure (Jones and Peterlin, 1994), by antisense RNA RNA interaction (Lee et al., 1993; Crespi et al., 1994), or by the association of two genomic-sense RNAs (Sit et al., 1998). To our knowledge, however, SRA is different from eukaryotic transcriptional coactivators in its ability to function as an RNA transcript to selectively regulate the activity of a family of transcriptional activators.

Functional evidence indicates that coregulators associate with nuclear receptors as members of multiprotein complexes (Rachez et al., 1998). It has been shown that hormone-activated receptors can recruit the coactivators of the SRC-1 family, the cointegrators p300 and CBP, histone acetyltransferase activity P/CAF, or chromatin-remodeling factors such as the human homologs of the yeast SWI/SNF proteins (reviewed by McKenna et al., 1999). In addition, multiple coregulators associate with each other (Kamei et al., 1996; Spencer et al., 1997; Torchia et al., 1997), and our laboratory has shown that distinct preformed complexes contain different subclasses of nuclear receptor coactivators in vivo (McKenna et al., 1998). We have provided biochemical evidence here that SRA elutes in a complex that also contains SRC-1 and that SRA was coimmunoprecipitated by SRC-1 and AR but not by p300, TR, RXR, or AF1-truncated AR in the absence of SRC-1. These observations raise the possibility that, through a specific association, SRA might function in part by modulating the activity of a distinct class of nuclear receptor coactivator complexes. Given its evident functional specificity, we favor a model in which SRA confers functional selectivity upon coactivator complexes recruited by liganded receptor, possibly acting as an adaptor molecule for type I receptors.

Taken together, our results have introduced an entirely novel concept, not only in nuclear receptor regulated transactivation, but in eukaryotic transcription as a whole. An RNA transcript, specifically expressed in steroid target tissues, functions as a component of a large multiprotein complex to selectively enhance transcriptional activation by steroid receptors. Regulation of transcription is a modular process, probably requiring different combinations of coregulators at different stages of transcription at different times. As an RNA transcript, subjected to rapid turnover and regulation, we envisage an important role for SRA in the dynamic process of transcription in which an activated receptor recruits diverse complexes mediating temporally and spatially distinct functions. Future studies will define more clearly the functional and physiological significance of this interesting eukaryotic transcriptional coregulator.

Experimental Procedures

DNA Library Screening

The coding sequence of the AF1 domain of the human PR_A (amino acids 165 567 of hPR_B) was subcloned into the pAS1 yeast expression plasmid in frame with the amino acid sequence of the GAL4-DBD (1 147). The yeast two-hybrid screen was performed as previously described in Onate et al. (1995). Transformants of a human B-lymphocyte cDNA expression library were tested in the Y190 yeast strain for interaction with progesterone-induced hPR_A. RACE was performed using the Marathon cDNA Amplification Kit (Clontech) with skeletal muscle mRNA (Clontech) and the following primers: − strand 5′-CTGGGGGATCCATCCTGGGGTGCG-3′ (On1), − strand 5′-CCTGCAGCAGTGCCAGGCGTCGG-3′ (On5), and + strand 5′-CGCGGCTGGAACGACCCGCCGC-3′ (On3). SRA clones were isolated by homology screening of human λgt11 cDNA libraries from skeletal muscle, heart, and HeLa S3 cells (Clontech), human genomic library EMBL3 SP6/T7 (Clontech), mouse heart cDNA library λZAP cDNA (Strategene), and 129SVJ mouse genomic library λgtFIX II (Stratagene) using bacteria strains and protocols as provided by

the library manufacturers. Both strands of SRA clones were sequenced using Sequenase (Amersham) or Thermal Cycle DNA Sequencing (New England Biolabs).

Northern and Southern Analysis

A human tissue Northern blot (MTN, Clontech) was hybridized with a probe corresponding to the NaeI HincII fragment of SRA. Tissue cell blots were prepared by isolation of total RNA using TRIzol Reagent (Life Technologies) and analyzed with a 1.5 kb probe corresponding to SRA isoform III (Figure 1A). The HeLa cell blot (Figure 5) was hybridized with a random labeled fragment of the firefly luciferase cDNA. The blots were stripped and subsequently hybridized with a probe specific for β-actin (MTN and tissue cell blots) or cyclophilin (HeLa cell blot), respectively. RT-PCR products were electrophoresed, blotted, and hybridized with a probe corresponding to isoform I of SRA. Probes were generated using random DNA labeling kit (Life Technologies) and 50 μCi of [α-^{32}P]dCTP, 300 Ci/mmol (ICN) followed by EtOH-precipitation or G-50 (Boehringer Mannheim) column purification.

Plasmids

The reporter constructs $(PRE)_2$-TATA-CAT and $(ERE)_2$-TATA-CAT have been described (Vegeto et al., 1992). The MMTV-Luc (Luc, luciferase) was generated by subcloning the Acc65I XbaI fragment from pGLBasic3 (Promega) into the blunt-ended EcoRI site of MMTV-KCR (Steve Chua, Baylor College of Medicine). The human CMV-driven mammalian expression vectors pSTC for human PR_B, GR, AR, and ER were generated by fusion of the cDNAs to the HSV-TK leader sequence containing a Kozak consensus sequence (Lanz et al., 1995); rat GR, GRΔAF1, and GR-DBD have been described (Rusconi and Yamamoto, 1987). PRΔAF1 is an N-terminal truncation of pSTC- hPR_B at the AccI site and re-ligation to the blunt-ended BamHI site of the TK leader; PRΔLBD is a C-terminal truncation of pSTC-hPR_B at the DraI site. TRβ, RARα, RARγ, RXRγ, Sp1, E2F, E47D, and CREB and corresponding CAT reporter constructs were from S. A. O. and M.-J. T. and published elsewhere (Cooney et al., 1992; Leng et al., 1994; Onate et al., 1995); PPARγ was a gift from Steven A. Kliewer (Glaxo Research Institute) and CBP from Richard Goodman (Vollum Institute, Oregon Health Sciences University). SRA-containing expression vectors were generated by subcloning the cDNAs into a modified linker of the CMV-driven pSCT-1 (Rusconi et al., 1990). Excision of BamHI fragment of pSCT-SRA and re-ligation generated ΔATG, and the fusion of the BamHI- or NaeI-restricted SRA to the HSV-TK leader sequence generated tk-ORF1 and tk-ORF2, respectively. The reading frame mutations ORF1, ORF2, and ORF3 were generated by PCR using the sense primers 5′-TGGGGGATCCTACCTCAGGTGCGG-3′, 5′-TGGGAGATCTATCC TAGGGTGCGG-3′, and 5′-TGGGGGATCCTACCTAGGGTGCGG-3′, followed by restriction subcloning into pSCT-SRA. YIle used the primer 5′-ATAGCAATTGGGCCTCCACCTCCTTCAAG-3′ to destroy an ATG and to introduce an MfeI site in mutant ORF2. Frameshift mutations were generated by restriction of SRA or mutant ORF2 with selected enzymes, filled in with Klenow DNA polymerase, and re-ligated at the following sites: BbsI (generated mutant B), SgrAI (S), MfeI and SgrAI (MS, YMS), and MfeI, BbsI, and SgrAI (YMBS). 3′ deletions at BbsI of ORF1, ORF2, and ORF3 generated ΔORF1, ΔORF2, and ΔORF3. All the vectors for in vitro transcription (Figure 6C) were generated by subcloning the cDNAs for SRA, SRC-1, AR, and Sp1 into MS2, which is a modified version of pSP64 poly(A) (Promega) containing an additional polylinker 3′ of the poly(A) sequence for linearization of the plasmid. ARΔAF1 was generated by subcloning of the HindIII XbaI fragment encoding amino acids 605 910 of pAR65 (Jenster et al., 1993) into MS2. pMS2-p300 was from J. W. (unpublished), and pMS2-TR and pMS2-RXR have been described (Wong et al., 1995).

Cell Culture and Transient Transfection Assays

Cell lines were routinely maintained at 37°C/5% CO_2 in Dulbecco's modified Eagles' medium (HeLa, COS) or RPMI medium 1640 (T-47D) supplemented with 5% 10% charcoal-stripped fetal calf serum. 10^5 cells were plated out per well in 12-well dishes for luciferase assays, 5×10^5 cells per well in 6-well dishes for CAT assays, and 10^6 cells per 10 cm dish for assays that involved cell culture in the presence of cycloheximide. Medium was replaced 3 hr prior to transfection with medium containing 50 μM cycloheximide and maintained until cell harvesting. Cells were transfected with the indicated DNAs using lipofectin (Life Technologies) or SuperFect (QIAGEN) and treated according to the manufacturer guidelines. In all transfection experiments, reporter plasmids were abundant (2.5 μg per 10^6 cells), whereas nuclear receptors were transfected in limiting amounts (20 100 ng per 10^6 cells). 2′-O-methoxyethyl ribose/2′-deoxyribose-stabilized oligonucleotides were generated by F. Bennett at ISIS Inc., Carlsbad, CA. Fifty to two hundred nanomoles of the antisense 5′-GGAACCGAGGATTATGAA-3′ and corresponding sense control were cotransfected and treated as described for plasmid DNA. Upon DNA addition, cells were cultured for 36 42 hr for CAT assays, 20 24 hr for luciferase assays, and 11 14 hr in the presence of cycloheximide. Ligand stimulation involved incubation of cells with progesterone (10 nM), RU486 (50 nM), dexamethasone (50 nM), R1881 (10 nM), or estradiol E2 (10 nM) for 6 8 hr prior to cell harvesting. Cell lysates were assayed for CAT activity with 100 μCi of [^{14}C]chloramphenicol and 5 mM acetyl coenzyme A (Sigma) as substrate and separated by thin-layer chromatography. Luciferase activity was determined using the luciferase assay system (Promega). Values were corrected for protein concentration. Data are presented as the mean (±SD) of triplicate values obtained from a representative experiment that was independently repeated at least three times.

Antibodies and Western Analysis

The mAbs against SRA, SRC-1, and hAR were prepared at the University of Colorado Health Science Center in collaboration with D. P. Edwards. SRA-mAb was raised against the peptide sequence TAEKNHTIPGFQQAS corresponding to the C terminus of the presumptive ORF1 of human SRA. The mAb was purified from hybridoma culture supernatants using a mAb TRAP GII column (Pharmacia). SRC-1-mAb was described previously (Spencer et al., 1997); AR-mAb recognizes the residues 299 315 of hAR and was a gift from N. Weigel (Baylor College of Medicine); the polyclonal Ab for AR-LBD (SP066; Kuiper et al., 1993) was a gift from J. Trapman and A. Brinkmann, Erasmus University, Rotterdam, The Netherlands; p300-Ab (Eckner et al., 1994) was a gift from R. Eckner, Molecular Biology, Zurich, and Sp1-Ab was obtained from Santa Cruz Biotechnology, Santa Cruz, CA. Immunoblotting was performed as described (Elashry-Stowers et al., 1988; Hanstein et al., 1996).

Gel Filtration

Biochemical fractionation of cell lysate on a Superose 6 gel filtration column (Pharmacia) was carried out as described (McKenna et al., 1998) except that cell lysates contained 1 2 U/μl RNasin ribonuclease inhibitor (Promega) and the columns were preequilibrated with RNasin. For antibody shift experiments, clarified lysates were preincubated at 4°C with 2 μg of SRC-1 mAb and a 4-fold excess of rabbit anti-mouse IgG (Zymed). Half of each column fraction (400 μl) was processed for RNA isolation and RT-PCR analysis, while the other portion was precipitated with BSA/trichloracetic acid, separated on 7.5% polyacrylamide gels, and transferred overnight to nitrocellulose membrane (BioRad) at 0°C 4 °C for Western analysis.

SRA-Specific RT-PCR

Cell extracts (20 30 μl) or column fractions (200 μl) were supplemented with 5 mM $MgSO_4$ and incubated for 25 min at 37°C with 20 40 U RNase-free DNaseI (Boehringer Mannheim) and 1 2 U/μl RNasin ribonuclease inhibitor (Promega). Total RNA was extracted using 1 ml TRIzol Reagent and processed according to the manufacturer's protocol (Life Technologies). EtOH-washed RNA was resuspended in 12 μl 2 pmol SRA-specific primers (On3: 5′-CGC GGCTGGAACGACCCGCCGC-3′ and On8: 5′-CAGACTCACCGGAC ACCATCTCCTA-3′; see Figure 2). First-strand cDNA synthesis was generated using Moloney reverse transcriptase and reagents supplied with the SuperScript II Kit (Life Technologies). Twenty percent (4 μl) of the reaction was used in a 50 μl PCR amplification using 5 U of Taq-DNA polymerase (Promega), 2 mM $MgCl_2$, 150 μM dNTPs, 1 μM of primers (On3/On8). PCR was performed as follows: 3 min at 95°C, 25 40 cycles of 30 s at 95 °C, 45 s at 58°C, 40 s at 71°C, and 5 min at 72°C. Alternatively, RNA from immunoprecipitations was processed by using the SuperScript One-Step RT-PCR System

(Life Technologies) and incubated at 50°C for 30 min prior to cDNA amplification. PCR products were visualized on 1.2% agarose/TAE gels and blotted to Zeta-Probe GT membrane (BioRad) by alkaline transfer for Southern analysis.

Immunoprecipitation in *Xenopus laevis* Oocytes

pP(A)LiSK-cDNA constructs were transcribed in vitro with SP6 RNA polymerase and the mMessage mMachine kit (Ambion) to generate 200 400 μg/μl specific mRNA. *Xenopus laevis* oocytes were injected with 27.6 nl specific mRNA and L-^{35}S-methionine and cultured for 12 16 hr at 18 °C in MBSH [10 mM HEPES (pH 7.6), 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.41 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$]. Oocytes were lysed in extract buffer (20 mM HEPES [pH 7.6], 70 mM KCl, 2 mM DTT, 0.1% NP-40, 8% Glycerol, 1 mM PMSF, and 1 U/μl RNasin) in a ratio of 10 μl extract buffer per oocyte. Clear lysates were incubated with 4 μg of SRC-1-mAb, 2 μg of AR-mAb, 8 μl of SP066 (AR-LBD Ab), 4 μg of rabbit polyclonal Sp1-Ab, or 4 μg of rabbit polyclonal TR-Ab together with a 4-fold excess of rabbit anti-mouse IgG (Zymed) for 35 min at 4°C followed by 30 min incubation at 4°C with protein A Sepharose (Pharmacia) that was washed and equilibrated in extract buffer. Subsequently, beads were washed five times with 5 vol of extract buffer, and bound material was analyzed by RT-PCR and by SDS-PAGE.

Acknowledgments

We thank Drs. Jeffrey M. Rosen and David Moore for helpful discussion; K. Bramlett and S. Chua for technical assistance; N. Weigel, A. Brinkmann, and R. Eckner for providing the antibodies mAR, SP066, and p300, respectively; G. Jenster for pAR65; S. Kliewer for PPARγ; R. Goodman for CBP; and F. Bennett (ISIS Inc.) for modified oligonucleotides. This work was supported by a Postdoctoral Fellowship from the Swiss National Science Foundation (R. B. L.), a United States Army Postdoctoral Breast Cancer Research Fellowship (N. J. M.), and grants from the National Institutes of Health (B. W. O.).

Received September 30, 1998; revised March 3, 1999.

References

Beato, M., and Sanchez-Pacheco, A. (1996). Interaction of steroid hormone receptors with the transcription initiation complex. Endocrine Rev. *17*, 587 609.

Bocquel, M.T., Kumar, V., Stricker, C., Chambon, P., and Gronemeyer, H. (1989). The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. Nucleic Acids Res. *17*, 2581 2595.

Chrivia, J.C., Kwok, R.P., Lamb, N., Hagiwara, M., Montminy, M.R., and Goodman, R.H. (1993). Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature *365*, 855 859.

Cooney, A.J., Tsai, S.Y., O'Malley, B.W., and Tsai, M.J. (1992). Chicken ovalbumin upstream promoter transcription factor (COUP-TF) dimers bind to different GGTCA response elements, allowing COUP-TF to repress hormonal induction of the vitamin D3, thyroid hormone, and retinoic acid receptors. Mol. Cell. Biol. *12*, 4153 4163.

Crespi, M.D., Jurkevitch, E., Poiret, M., d'Aubenton-Carafa, Y., Petrovics, G., Kondorosi, E., and Kondorosi, A. (1994). enod40, a gene expressed during nodule organogenesis, codes for a non-translatable RNA involved in plant growth. EMBO J. *13*, 5099 5112.

Danielian, P.S., White, R., Lees, J.A., and Parker, M.G. (1992). Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. EMBO J. *11*, 1025 1033.

Eckner, R., Ewen, M.E., Newsome, D., Gerdes, M., DeCaprio, J.A., Lawrence, J.B., and Livingston, D.M. (1994). Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev. *8*, 869 884.

Elashry-Stowers, D., Zava, D.T., Speers, W.C., and Edwards, D.P. (1988). Immunocytochemical localization of progesterone receptors in breast cancer with anti-human receptor monoclonal antibodies. Cancer Res. *48*, 6462 6474.

Halachmi, S., Marden, E., Martin, G., MacKay, H., Abbondanza, C., and Brown, M. (1994). Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. Science *264*, 1455 1458.

Hanstein, B., Eckner, R., DiRenzo, J., Halachmi, S., Liu, H., Searcy, B., Kurokawa, R., and Brown, M. (1996). p300 is a component of an estrogen receptor coactivator complex. Proc. Natl. Acad. Sci. USA *93*, 11540 11545.

Horwitz, K.B., Jackson, T.A., Bain, D.L., Richer, J.K., Takimoto, G.S., and Tung, L. (1996). Nuclear receptor coactivators and corepressors. Mol. Endocrinol. *10*, 1167 1177.

Jaeger, L. (1997). The new world of ribozymes. Curr. Opin. Struct. Biol. *7*, 324 335.

Jenster, G., Trapman, J., and Brinkmann, A.O. (1993). Nuclear import of the human androgen receptor. Biochem. J. *293*, 761 768.

Jonat, C., Rahmsdorf, H.J., Park, K.K., Cato, A.C., Gebel, S., Ponta, H., and Herrlich, P. (1990). Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell *62*, 1189 1204.

Jones, K.A., and Peterlin, B.M. (1994). Control of RNA initiation and elongation at the HIV-1 promoter. Annu. Rev. Biochem. *63*, 717 743.

Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., Glass, C.K., and Rosenfeld, M.G. (1996). A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell *85*, 403 414.

Kuiper, G.G., de Ruiter, P.E., Trapman, J., Jenster, G., and Brinkmann, A.O. (1993). In vitro translation of androgen receptor cRNA results in an activated androgen receptor protein. Biochem. J. *296*, 161 167.

Lanz, R.B., and Rusconi, S. (1994). A conserved carboxy-terminal subdomain is important for ligand interpretation and transactivation by nuclear receptors. Endocrinology *135*, 2183 2195.

Lanz, R.B., Wieland, S., Hug, M., and Rusconi, S. (1995). A transcriptional repressor obtained by alternative translation of a trinucleotide repeat. Nucleic Acids Res. *23*, 138 145.

Lee, R.C., Feinbaum, R.L., and Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell *75*, 843 854.

Leng, X., Blanco, J., Tsai, S.Y., Ozato, K., O'Malley, B.W., and Tsai, M.J. (1994). Mechanisms for synergistic activation of thyroid hormone receptor and retinoid X receptor on different response elements. J. Biol. Chem. *269*, 31436 31442.

Mangelsdorf, D.J., and Evans, R.M. (1995). The RXR heterodimers and orphan receptors. Cell *83*, 841 850.

McKenna, N.J., Nawaz, Z., Tsai, S.Y., Tsai, M.J., and O'Malley, B.W. (1998). Distinct steady-state nuclear receptor coregulator complexes exist in vivo. Proc. Natl. Acad. Sci. USA *95*, 11697 11702.

McKenna, N.J., Lanz, R.B., and O'Malley, B.W. (1999). Nuclear receptor coregulators: cellular and molecular biology. Endocrine Rev., in press.

Meyer, M.E., Gronemeyer, H., Turcotte, B., Bocquel, M.T., Tasset, D., and Chambon, P. (1989). Steroid hormone receptors compete for factors that mediate their enhancer function. Cell *57*, 433 442.

Onate, S.A., Tsai, S.Y., Tsai, M.J., and O'Malley, B.W. (1995). Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science *270*, 1354 1357.

Rachez, C., Suldan, Z., Ward, J., Chang, C.P., Burakov, D., Erdjument-Bromage, H., Tempst, P., and Freedman, L.P. (1998). A novel protein complex that interacts with the vitamin D3 receptor in a ligand-dependent manner and enhances VDR transactivation in a cell-free system. Genes Dev. *12*, 1787 1800.

Rusconi, S., and Yamamoto, K.R. (1987). Functional dissection of the hormone and DNA binding activities of the glucocorticoid receptor. EMBO J. *6*, 1309 1315.

Rusconi, S., Severne, Y., Georgiev, O., Galli, I., and Wieland, S. (1990). A novel expression assay to study transcriptional activators. Gene *89*, 211 221.

Sit, T.L., Vaewhongs, A.A., and Lommel, S.A. (1998). RNA-mediated trans-activation of transcription from a viral RNA. Science *281*, 829 832.

Spencer, T.E., Jenster, G., Burcin, M.M., Allis, C.D., Zhou, J.X., Mizzen, C.A., McKenna, N.J., Onate, S.A., Tsai, S.Y., Tsai, M.J., and O'Malley, B.W. (1997). Steroid receptor coactivator-1 is a histone acetyltransferase. Nature *389*, 194 198.

Suen, C.S., Berrodin, T.J., Mastroeni, R., Cheskis, B.J., Lyttle, C.R., and Frail, D.E. (1998). A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. J. Biol. Chem. *273*, 27645 27653.

Tasset, D., Tora, L., Fromental, C., Scheer, E., and Chambon, P. (1990). Distinct classes of transcriptional activating domains function by different mechanisms. Cell *62*, 1177 1187.

Tora, L., Gronemeyer, H., Turcotte, B., Gaub, M.P., and Chambon, P. (1988). The N-terminal region of the chicken progesterone receptor specifies target gene activation. Nature *333*, 185 188.

Torchia, J., Rose, D.W., Inostroza, J., Kamei, Y., Westin, S., Glass, C.K., and Rosenfeld, M.G. (1997). The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. Nature *387*, 677 684.

Tsai, M.J., and O'Malley, B.W. (1994). Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu. Rev. Biochem. *63*, 451 486.

Tsai, S.Y., Sagami, I., Wang, H., Tsai, M.J., and O'Malley, B.W. (1987). Interactions between a DNA-binding transcription factor (COUP) and a non-DNA binding factor (S300-II). Cell *50*, 701 709.

Vegeto, E., Allan, G.F., Schrader, W.T., Tsai, M.J., McDonnell, D.P., and O'Malley, B.W. (1992). The mechanism of RU486 antagonism is dependent on the conformation of the carboxy-terminal tail of the human progesterone receptor. Cell *69*, 703 713.

Wong, J., Shi, Y.B., and Wolffe, A.P. (1995). A role for nucleosome assembly in both silencing and activation of the Xenopus TR beta A gene by the thyroid hormone receptor. Genes Dev. *9*, 2696 2711.

Xu, J.M., Qiu, Y.H., Demayo, F.J., Tsai, S.Y., Tsai, M.J., and O'Malley, B.W. (1998). Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science *279*, 1922 1925.

Yoshinaga, S.K., Peterson, C.L., Herskowitz, I., and Yamamoto, K.R. (1992). Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science *258*, 1598 1604.

Zhang, B., and Cech, T.R. (1997). Peptide bond formation by in vitro selected ribozymes. Nature *390*, 96 100.

GenBank Accession Numbers

The GenBank accession numbers for the human SRA and mouse SRA reported in this paper are AF092038 and AF092039, respectively.

0163-769X/99/$03.00/0
Endocrine Reviews 20(3): 321–344
Copyright © 1999 by The Endocrine Society
Printed in U.S.A.

Nuclear Receptor Coregulators: Cellular and Molecular Biology*

NEIL J. McKENNA, RAINER B. LANZ, AND BERT W. O'MALLEY

Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

I. Introduction

NUCLEAR receptor coregulators are coactivators or corepressors that are required by nuclear receptors for efficient transcriptional regulation. In this context, we define coactivators, broadly, as molecules that interact with nuclear receptors and enhance their transactivation. Analogously, we refer to nuclear receptor corepressors as factors that interact with nuclear receptors and lower the transcription rate at their target genes. Most coregulators are, by definition, rate limiting for nuclear receptor activation and repression, but do not significantly alter basal transcription. Recent data have indicated multiple modes of action of coregulators, including direct interactions with basal transcription factors and covalent modification of histones and other proteins. Reflecting this functional diversity, many coregulators exist in distinct steady state precomplexes, which are thought to associate in promoter-specific configurations. In addition, these factors may function as molecular gates to enable integration of diverse signal transduction pathways at nuclear receptor-regulated promoters. This review will summarize selected aspects of our current knowledge of the cellular and molecular biology of nuclear receptor coregulators.

Address reprint requests to: Bert W. O'Malley, M.D., Department of Cell Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030 USA. E-mail: berto@bcm.tmc.edu

* Research described in this review was supported by a United States Army Breast Cancer Postdoctoral Award (to N.J.McK.); a Swiss National Foundation Fellowship (to R.B.L.); and by NIH and NICHD Grants (to B.W.O.).

A. The nuclear receptor superfamily

Nuclear receptors are ligand-inducible transcription factors that specifically regulate the expression of target genes involved in metabolism, development, and reproduction. Their primary function is to mediate the transcriptional response in target cells to hormones such as the sex steroids (progestins, estrogens, and androgens), adrenal steroids (glucocorticoids and mineralocorticoids), vitamin D_3, and thyroid and retinoid (9-*cis* and all-*trans*) hormones, in addition to a variety of other metabolic ligands. More than 100 nuclear receptors are known to exist, and, together, these proteins comprise the single largest family of metazoan transcription factors, the nuclear receptor superfamily.

Even the briefest consideration of research on the nuclear receptor superfamily affords an appreciation of its global importance in cellular signaling and differentiation. Seminal studies in the 1960s identified the estrogen receptor (ER), and the general pathway for steroid hormone action was subsequently elucidated. Numerous subsequent studies led to the belief that steroid receptors act at the level of DNA to enhance recruitment of the preinitiation complex of general transcription factors (GTFs) at target promoters. The cloning in the mid- to late 1980s of cDNAs encoding many of the receptors prefaced their designation, on the basis of extensive amino acid sequence identity, as an evolutionarily related family of proteins. Phylogenetic analysis has identified several subfamilies within this superfamily: type I ("classical" or "steroid") receptors include those for progestins (PR), estrogens (ER), androgens (AR), glucocorticoids (GR), and mineralocorticoids (MR), whereas type II receptors encompass those for thyroid hormone (TR), all-*trans* retinoic acid (RAR), 9-*cis* retinoic acid (RXR), and vitamin D_3 (VDR). A third subclass contains orphan receptors, for which ligands are only now being characterized. Although they have common structural features, divergence of the steroid and thyroid/retinoid/vitamin D_3 receptor subclasses is supported by differences in their functional characteristics, as well as by their discrepant recognition of *cis*-acting hormone response elements. Type I receptors, in the absence of ligand, are sequestered in nonproductive associations with heat shock proteins and, in this state, are not thought to influence the rate of transcription of their cognate promoters. Conversely, type II receptors are able to bind DNA in the absence of ligand and often exert a repressive effect upon the activity of their subject promoters, a phenomenon referred to as silencing (1). Type I receptors bind to palindromic repeats in a homodimeric head-to-head arrangement only in the presence of ligand, whereas type II

receptors bind constitutively to response elements that contain direct repeats. In addition, type II receptors exhibit promiscuous dimerization patterns, many involving heterodimerization with RXR, and such interactions may serve to modulate the amplitude of the transcriptional response to ligand.

Meticulous domain-mapping experiments have identified a number of functional domains now designated as defining structural features of members of the nuclear receptor superfamily. For a detailed discussion of these domains, the reader is referred to Tsai and O'Malley (1) and references therein. Broadly, the receptor structure is comprised of: an amino-terminal activation function, AF-1 (A/B domain); the DNA-binding domain (DBD) (C); a hinge region (D); and a carboxy-terminal ligand-binding domain (LBD) (E). Mutational analysis of the E domain led to the designation of a second activation function, AF-2, which is indispensable for proper ligand-dependent activation by nuclear receptors (2–4). Other functions have been ascribed to the E domain, including ligand binding (5), heat shock protein (hsp) interactions (6), and nuclear localization (7). These functional domains reflect a intricate, but well characterized, ligand-mediated receptor activation pathway (Fig. 1).This multistep process involves activation of receptor by binding of the cognate hormone, a change in receptor structure and dissociation of several heat shock proteins, nuclear translocation of the activated receptor (in the case of GR, MR, AR, and PR), and dimerization and apposition of the transformed receptor to its DNA response elements. Rather less well characterized though, is the sequence of events by which the activated, DNA-bound receptor achieves transcriptional regulation. While the role of GTFs in mediating basal transcription is well documented (see *Section I.B.* below), it has recently become clear that nuclear receptors recruit a host of ancillary factors (coregulators) that 1) create, depending upon the activation state of the receptor, a transcriptionally permissive, or nonpermissive environment at the promoter and 2) communicate with the GTFs and RNA Pol II.

B. General transcription factors (GTFs)

The entire sequence of events leading to the assembly of a preinitiation complex of GTFs at enhancer-controlled promoters is beyond the scope of this chapter. For a thorough discussion of eukaryotic transcriptional initiation, the reader is referred to selected reviews (8, 9). Steroid and thyroid/retinoid hormones regulate transcription via enhancer elements that may be several kilobases from their target promoters, at which transcription is mediated by RNA polymerase II (Pol II). The initial step is the binding of TFIID to the promoter at a short distance from the transcriptional start site. TFIID functions as a multiprotein complex composed of TATA-binding protein (TBP) and the highly conserved TBP-associated factors (TAF_{II}s). Human TFIID has been shown to be comprised of at least two distinct subpopulations: a core group containing human (h)$TAF_{II}250$, $hTAF_{II}135$, $hTAF_{II}100$, and $hTAF_{II}28$, present in all TFIID complexes; and another group containing promoter-specific $hTAF_{II}$s, such as $hTAF_{II}30$, $hTAF_{II}20$, and $hTAF_{II}18$ (10, 11). After TFIID binding is that of TFIIB, a GTF with affinity for single-stranded DNA, which apposes to sequences adjacent to the TATA box in response to a critical change in DNA topology induced by TBP (12). Recruitment by TFIIB of another GTF, TFIIF-α, is followed by binding of RNA Pol II (13). While this description implies a stepwise accretion of factors, recent evidence suggests that stable, preformed basal transcription complexes may also exist, which contain RNA Pol II in addition to other GTFs (14). Ultimately, it is by influencing the rate of assembly of such complexes that nuclear receptors, in association with their coregulators, achieve transcriptional regulation at hormone-regulated promoters.

II. Nuclear Receptor Coactivators

A. Background

1. Direct interactions between receptors and GTFs. Direct protein-protein interactions, the functionality of which is yet to be determined, have been reported between receptors and GTFs. TBP and several TAF_{II}s interact functionally with specific receptors and are, by our definition, nuclear receptor coactivators. Consistent with the designation of TBP recruitment as a rate-limiting step in transcriptional initiation (15), several interactions between TBP and nuclear receptors have been reported. Protein-protein interaction assays, such as the yeast two-hybrid screen and *in vitro* binding assays with recombinant proteins, have detected an association between a portion of the TBP and the AF-2 function of RXR (16). Similarly, AF-1 and AF-2 of the ER bind TBP *in vitro* (17), and a similar interaction has been documented between PR and the $TAF_{II}110$ subunit of TFIID (18). In addition to those with TBP, contacts of nuclear receptors with other GTFs have been described. Interactions between AR and TFIIF (19) and RAR and TFIIH (20), and the interactions of TFIIB with VDR (21) and other nuclear receptors (22), may be influential in modulating a DNA-bound ternary complex of receptor, TFIIB, and TBP-TAF_{II}s. These interactions suggest that direct inter-

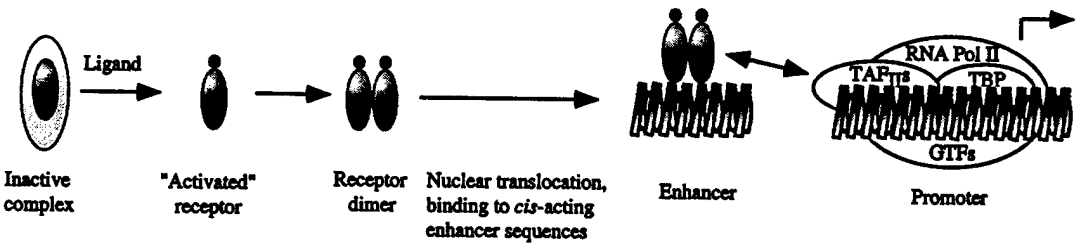

FIG. 1. Model for transactivation by a nuclear receptor. While this model applies generally to type I receptors, type II receptors can bind their response elements in the absence of ligand.

actions between nuclear receptors and GTFs may contribute to the assembly of final transcriptional complexes at their target promoters.

2. Evidence of the existence of coactivators. An early indication of the interaction of activated receptors with factors other than GTFs was the phenomenon of squelching, or transcriptional interference between receptors, in transient receptor/reporter co-transfection assays (23, 24). In the context of activation, squelching defines the reduction in transactivation of a promoter regulated by nuclear receptor A (more specifically, an activation function) in the presence of a distinct, activated receptor B. The clear inference from such experiments was that titration of a cellular pool of factors for which the activation functions competed limited the overall reporter gene activity of the receptors. Such experiments indicated that common cofactors might be an important functional link between the receptor and transcriptional initiation. Supportive of such a notion was the fact that tissue- and promoter specificity were characteristic of the activation functions of the ER (25) and RAR (26). Collectively, these studies suggested a level of control at enhancer-controlled promoters beyond the actual receptor-response element interaction.

B. Receptor-associated proteins and coactivators

1. ER-associated proteins (ERAPs) and RIPs. In a seminal study, Halachmi *et al.* (27) used a purified ligand-bound ER LBD to identify ER-interacting proteins from ^{35}S-radiolabeled MCF-7 cell lysates. Two proteins, ERAP-140 and ERAP-160, were identified in this manner. A potential role for these proteins in ER function was suggested both by the ligand dependence of their interaction with ER and by the fact that transcriptionally defective mutants of ER failed to recruit these factors. Moreover, the estrogen antagonists 4-hydroxytamoxifen (4-HT) and the pure antiestrogen, ICI 164384, uncoupled the ER-ERAP interaction (27). While ERAP-140 and ERAP-160 (subsequently cloned as SRC-1/hSRC-1[1], see *Section II.B.2.a*) exhibited similar associations with RARα and RXRβ, other transcriptional activators, including Rb and Pit-1, did not interact with the ERAPs, indicating a degree of specificity in ERAP binding. Eggert *et al.* (28) biochemically characterized a 170-kDa protein, GRIP-170 (GR-interacting protein 170, postulated to be equivalent to ERAP-160), which interacted with GR in a hormone-dependent manner and which was enriched in a mammalian cellular fraction that potentiated GR activity in an *in vitro* transactivation assay.

Cavaillès *et al.* (29) used far-Western blotting and *in vitro* interaction assays to identify receptor-interacting proteins (RIPs) of 160, 140, and 80 kDa. As with ERAPs, RIPs failed to interact either with antiestrogen-bound ER or with transcriptionally-defective mutants of ER. Subsequently, this group (30) reported the cloning of the cDNA encoding RIP-140 and demonstrated its widespread expression in mammalian tissues. *In vitro* interactions of RIP-140 were demonstrated with wild-type ER, but not with transcriptionally defective ER mutants. Although marginal coactivation of ligand-dependent ER transactivation was exhibited in transient cotransfection in mammalian cells, no interaction of RIP-140 with GTFs such as TBP or TFIIB could be demonstrated. Indeed, recent evidence, while supporting the ligand-dependent interaction of RIP-140 with TR2, suggests that RIP140 acts as a corepressor for this orphan receptor member of the nuclear receptor superfamily (31).

2. The SRC family. Table 1 shows a summary of the properties of characterized nuclear receptor coactivators. To encourage brevity, consensus, and clarity in discussion of SRC coactivators, we are adopting the proposed nomenclature[1] (32).

a. SRC-1/NCoA-1. The cloning and characterization of steroid receptor-coactivator-1 (SRC-1/hSRC-1) by our laboratory (33) was the first description of an authentic common transcriptional mediator for nuclear receptors. Identified using a yeast two-hybrid screen of a human B-lymphocyte cDNA library with a bait encoding the PR LBD, hSRC-1 exhibits a broad range of specificity in the coactivation of the ligand-dependent transactivation of nuclear receptors, including PR, GR, ER, TR, RXR (33), HNF-4 (hepatocyte nuclear factor 4; Ref. 34), and PPARγ (peroxisome proliferator-activated receptor; Ref. 35). The interaction of hSRC-1 with the PR LBD is ligand dependent (33) and is abolished in the presence of the antiprogestin RU486. Furthermore, hSRC-1 has been shown to be capable of reversing the squelching of PR transactivation by cotransfected ER, indicating that it constitutes a common, limiting factor recruited by the LBDs of ER and PR for efficient transactivation (see *Section II.A*). In addition, a hSRC-1 mutant, containing only the C-terminal receptor-interacting domain (Fig. 2), suppresses PR coactivation by hSRC-1 in a dominant-negative fashion, both in transient transfection (33) and by *in vitro* transcription assay (36). Lee and colleagues have shown that, in addition to nuclear receptors, hSRC-1 modestly coactivates other transcription factors, including AP-1 (37), serum response factor (38), and NF-κB (39).

Several studies have indicated the ability of hSRC-1 to mediate functional interactions between the N-terminal AF-1 and C-terminal AF-2 activation functions of steroid receptors. Individual domains of hSRC-1 are required for full functional synergy between AF-1 and AF-2 of the PR (40), as well as ER (41) and AR (42), indicating that the efficient assembly of a preinitiation complex by steroid receptors is contingent, at least in part, on an SRC-1-assisted interaction between their individual AFs. Because the functional interaction of SRC-1 with receptors appears to be largely dependent on the integrity of a conserved amphipathic helix in the AF-2 region of receptors (43), we suggest that it and other predominantly AF-2 interacting coactivators be referred to as

[1] To resolve the complex issue of nomenclature in this family, we are adopting a unifying system proposed by Li and Chen (32). The prefix "h" will be used for all human clones and the prefix "m" will identify those clones originating in the mouse. The family will be called the SRC coactivator family to acknowledge the initial cloning of SRC-1 (33). The name hSRC-1 will identify SRC-1 (33); and the name mSRC-1 will represent NCoA-1 (45). GRIP1 (48) and NCoA-2 (50) will be referred to as mSRC-2; and hSRC-2 will represent TIF2 (47). RAC3 (54)/ACTR (53)/AIB1 (55)/TRAM-1 (56)/SRC-3 (57) will be referred to as hSRC-3; and p/CIP will be identified as mSRC-3. Throughout this review, discussions of individual clones will refer to original clone name/name under proposed nomenclature, *e.g.*, NCoA-1/mSRC-1.

TABLE 1. Nuclear receptor coactivators

Cofactor	Alternative designations	Related factors	Comments	References
ERAP-160	GRIP-170, p160	ERAP-140	ERAPs bind ER in ligand-dependent manner; mammalian cellular fraction enriched in GRIP-170 coactivates GR.	27, 28
RIP-140		RIP-160	Interacts with and coactivates ER; acts as a corepressor for TR2 orphan receptor.	29, 30, 31
TBP/TAF_{II}s			Interact with and specifically coactivate nuclear receptors.	10, 16–22, 85–86
SRC-1	hSRC-1 NCoA-1/mSRC-1 p160	TIF2/hSRC-2 p/CIP/mSRC-3 hSRC-3	Interacts with and coactivates nuclear receptors; interacts with CBP/p300; contacts basal transcription factors; possesses acetyltransferase activity, interacts with PCAF; contains autonomous activation domains; targeted deletion causes partial hormone insensitivity in mice.	33–46, 58–60, 67, 93, 104, 105, 109
Trip-1	Sug-1	Trips	Substitutes for Sug1 in yeast and interacts with TR, Gal4 and VP16; Trips interact with RXR and show homology to yeast transcriptional activators; Sug1 interacts with RARα in yeast and mammalian cells; contains ATPase domain.	74, 75
TIF1α		TIF1β, γ	Interacts with and coactivates RXR/RAR AF2 in yeast; TIF-1α represses when fused to DNA-binding domain; TIF-1s interact with factors related to chromatin-modifying proteins.	187–194
ARA-70			ARA70 interacts with and coactivates AR in prostate cells.	94–95
TRAPs	DRIPs		Biochemically-identified protein complexes; interact with liganded TR/VDR; enhance TR and VDR-mediated transcription *in vitro*. TRAP/DRIP220 contains NR boxes; share components with SMCC complex.	76–80
CBP		p300	Interacts with and coactivates multiple activators, including nuclear receptors; acetyltransferase; interacts with PCAF, SRC-1, TIF2/hSRC-2 and p/CIP mSRC-3; mutated in Rubinstein/Taybi syndrome.	45, 50, 51, 58, 60, 81, 99, 100, 103–106, 165, 171–173, 175
p300		CBP	Broad functional similarity to CBP; interacts with and coactivates nuclear receptors; possesses acetyltransferase activity; interacts with PCAF, SRC-1 and p/CIP/mSRC-3.	100–102, 107–112, 165, 174
PCAF	hGCN5		Possesses intrinsic acetyltransferase activity; interacts with PR and SRC-1; interacts with TR and ACTR; present in 2 MDa complex containing histone-like TAF_{II}s.	36, 58, 165, 167–169
TIF2/hSRC-2	GRIP-1/mSRC-2 NCoA-2, p160	hSRC-1 mSRC-3/hSRC-3	Interacts with and coactivates nuclear receptors; interacts with CBP.	47–52, 58
TRIP230			Rb-binding protein that selectively coactivates TR in specific cell types.	96
L7/SPA			Interacts with RU486-bound PR and enhances partial agonist activity of RU486 with PR.	71
p/CIP/mSRC-3	ACTR/hSRC-3 RAC3/hSRC-3 AIB-1/hSRC TRAM-1/hSRC-3 p160, SRC-3	SRC-1 TIF2/hSRC-2 GRIP-1/mSRC-2	p/CIP coactivates CBP-mediated signaling pathways; interacts with CBP and p300. ACTR interacts with and coactivates TR; possesses acetyltransferase activity; interacts with CBP/p300 and PCAF. RAC3 interacts with nuclear receptors, coactivates PR & RAR and contains autonomous activation domain. AIB-1 interacts with, coactivates ER; overexpressed in breast tumors and breast cancer cell lines. TRAM-1 interacts with and coactivates TR. SRC-3 preferentially coactivates ERα over ERβ	50 53 54 55 56 57

TABLE 1. Nuclear receptor coactivators—*continued*

Cofactor	Alternative designations	Related factors	Comments	References
E6-AP		RPF-1 (hRSP5)	E3 ubiquitin-protein ligases; E6-AP interacts with and coactivates AR, ER, PR and GR; E3 ubiquitin ligase; E6-AP and RPF-1 synergistically enhance PR activation.	58, 68–70
BRG-1	SWI2/SNF2	Brahma	Interacts with GR and ER; required by GR for chromatin remodeling.	176–186
NSD-1			SET domain-containing protein; interacts with liganded and unliganded nuclear receptor LBDs.	63
PGC-1			Expressed in brown adipose tissue and skeletal muscle; interacts with PPAR-γ in a ligand-independent manner; induced at low temperatures, enhances PPAR-γ activation during adaptive thermogenesis.	97
HMG-1		HMG-2	Coactivation specific for steroid receptors; promotes DNA binding by steroid receptors.	98
NCoA-62		BX42	Interacts with VDR, RAR and RXR; coactivates VDR strongly, GR, RAR and ER weakly.	89
TSC-2	Tuberin		Interacts with RXR; coactivates PPARγ and VDR; gene located in a locus, 16p13, associated with familial tuberous sclerosis.	90–92
PBP	TRAP 220, TRIP2, mPIP9		PBP binds to PPARγ, RARα, RXR and TRβ-1 and coactivates PPARγ; originally isolated as a TR-binding protein, TRIP2.	77, 79, 87, 88
Positive cofactors	PC2, PC4		Synergistically activate TR-TRAP activation in an *in vitro* transcription assay.	82
ADA			Yeast acetylase complex which mediates AF-2 dependent activation by RXR and ER.	166
SMCC		TRAPs/DRIPs	Human SRB/mediator-containing complex; contains components homologous to TRAPs/DRIPs.	80
SRA			Functions as RNA transcript; selectively coactivates AF-1 of steroid receptors; present in SRC-1 complex.	93
SNURF			Interacts with AR in yeast and mammalian cells; coactivates AR, PR, GR, Sp1 and AP-1 and basal activity; contains RING finger domain.	213
ARIP3		Miz1, PIAS3, GBP	Interacts with and modestly coactivates AR; expression specific to the testis in humans.	214

SRC family members are referred to by original clone names/names according to proposed nomenclature (Ref. 32; see footnote 1).

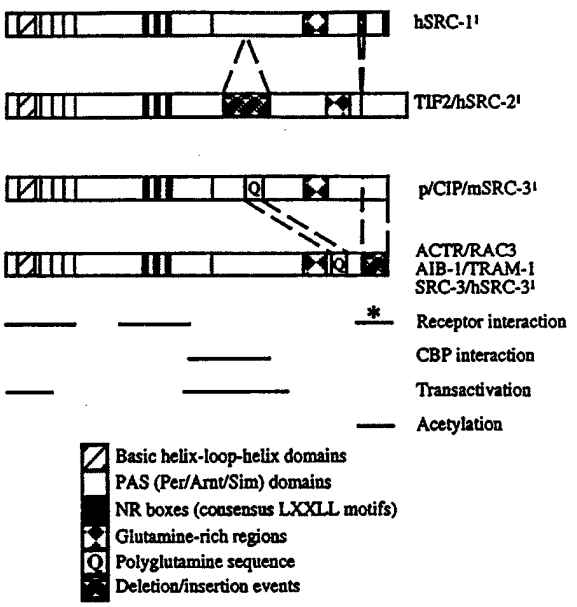

FIG. 2. Multiple members of the SRC family. Proteins have been aligned according to major structural similarities and to emphasize both the structural divergence of the carboxy termini and the conservation of the amino-terminal domains of SRC family members. Regions to which specific functions of individual coactivators have been assigned are indicated. We have adopted the unifying nomenclature of Li and Chen (32). m, Mouse; h, human. *, Only SRC-1 contains a consensus LXXLL/NR box motif in this region.

AF-2 coactivators, to distinguish them from non-AF-2 interacting factors, such as steroid receptor RNA activator (SRA; *Section II.B.4.a*) and PPARγ coactivator-1 (PGC-1, *Section II.B.4.d*).

SRC-1 contains two activation domains that retain their activity when transferred to a heterologous DBD (40) and, interestingly, Takeshita *et al.* (44) have demonstrated the interaction of hSRC-1 *in vitro* with TFIIB and TBP. When a longer form of SRC-1 (45) was cloned in the mouse [NCoA-1 (nuclear receptor coactivator 1)/mSRC-1], it was found to contain an additional 380 amino-terminal residues relative to the initial SRC-1 clone (33), which might have represented either a partial clone or a splice variant of the full length protein. Sequence analysis of the amino-terminal region has identified tandem bHLH (for basic helix-loop-helix) and PAS (for Per/Arnt/Sim homology) domains. The bHLH/PAS domains mediate homodimeric and heterodimeric interactions between proteins containing these motifs (46), and their conservation in the SRC family (see *Section II.B.2.d*, Fig. 2) suggests that functional cross-talk between nuclear receptor-mediated pathways and other PAS-containing factors might occur (45). On the basis of differences in the deduced encoded amino acid sequences of cDNA clones isolated during screens, the existence of splicing variants of NCoA-1/mSRC-1 has been conjectured (45), but their biological role, if any, is unknown at present.

b. GRIP1/TIF2/NCoA-2/SRC-2.[1] Characterization of cDNAs encoding GRIP1 (GR-interacting protein 1), TIF2 (transcription intermediary factor 2), and NCoA-2, 160-kDa nuclear receptor-interacting proteins with considerable sequence and functional similarity to SRC-1 (47–50), established the existence of what is now termed the SRC family (Fig. 2), also referred to previously as the p160 family (45). GRIP1 (mSRC-2) and TIF2 (hSRC-2) associate in a ligand-dependent manner *in vitro* with several receptor LBDs (47) and, *in vivo*, with RARα, ER, and PR in the presence of hormone, but not hormonal antagonists (47–49). In addition, GRIP1/mSRC-2 and TIF2/hSRC-2 contain two autonomous activation domains capable of stimulating transcription when tethered to a heterologous DBD in yeast (48) and in mammalian cells (47, 48, 51). Furthermore, overexpression of TIF2, like SRC-1/hSRC-1, is capable of relieving squelching by ER (47). Furthermore, a truncated GRIP1/mSRC-2 inhibits hormone-dependent expression from the mouse mammary tumor virus (MMTV) promoter, a property reminiscent of the dominant-negative properties of the receptor-interacting domain of SRC-1/hSRC-1 in relation to PR transactivation (48). GRIP1/mSRC-2 is also capable of enhancing transactivation in yeast of fusions of type I and type II receptors with the DBD of the yeast Gal4 activator (49, 52).

c. p/CIP/RAC3/ACTR/AIB-1/TRAM-1/SRC-3.[1] The identification of a third member of the SRC/p160 family, a highly polymorphic protein isolated independently as p/CIP [p300/CBP cointegrator-associated protein (50)], ACTR [activator of thyroid receptor (53)], RAC-3 [receptor-associated coactivator 3 (54)], AIB-1 [amplified in breast cancer-1 (55)], TRAM-1 [thyroid receptor activator molecule 1 (56)] and SRC-3 (57), serves to illustrate the growing complexity of nomenclature in the SRC family. For clarity, the unifying term "SRC-3" has been proposed for this member of the SRC family. hSRC-3 interacts with and coactivates a wide variety of nuclear receptors in a ligand-dependent manner, including RAR, TR, RXR, GR (53), PR (54), and ER (55). p/CIP/mSRC-3, however, exhibits greater promiscuity than other SRC family members by enhancing the transcriptional activity of a number of different activators, including interferon-α and cAMP regulatory element binding protein (CREB; Ref. 50), which were previously shown to be primarily dependent upon the transcriptional cointegrator CREB-binding protein (CBP; *Section II.B.5*) for efficient activation. Furthermore, SRC-3 selectively enhances the transcriptional activity of ERα over that of ERβ, possibly reflecting a 60% difference in homology between the LBDs of these isoforms (57). Li *et al.* (54) have demonstrated a feed-forward mechanism for regulation of RAC3/hSRC-3 expression by retinoid treatment in HL-60 cells, adding an additional level of control to nuclear receptor action.

d. Redundancy and diversity in the SRC family. The SRC family (Fig. 2) is defined by an overall sequence similarity of 40% between the three proteins, distinguishing its members from other coregulator classes, such as the p300/CBP cointegrators (*Section II.B.5*), E3 ubiquitin-protein ligases (*Section II.B.3.a*), TRAPs (TR-associated proteins, *Section II.B.3.e*), and the TIF-1 family (transcriptional intermediary factor-1, *Section IV.D.2*). The extent of sequence conservation between individual members is most apparent in their N-terminal domains, in which the bHLH/PAS domains exhibit a high degree of similarity. The extensive homology among SRC family members in this region is unique among PAS-containing proteins (54), identifying these proteins as a distinct subfamily of PAS factors. Like other PAS proteins, evidence

suggests that SRC family members are capable of forming heteromultimeric and homomultimeric complexes *in vivo* (58), although the requirement of the PAS domain for such interactions, as well as their functional significance, is unclear.

Redundancy within the SRC family is indicated by the phenotype arising from targeted deletion of the murine *SRC-1* locus. In this study, our laboratory provided the first *in vivo* data for the biological role of mSRC-1 expression in hormone-responsive pathways involved in adult sexual maturation. The phenotype of the SRC-1 null mutant is characterized by viability and fertility of both sexes against a background of significantly decreased growth of steroid target organs such as prostate, testis, and mammary gland in response to hormonal stimulation (59). We believe that the phenotype of the mSRC-1 null mutant arises in part from the compensatory overexpression of GRIP1/mTIF2 in certain tissues in the mutant, providing *in vivo* evidence of partial functional redundancy between mSRC-1 and GRIP1/mTIF2.

While the considerable sequence similarity between SRC family members indicates some redundancy of function, there is sufficient sequence divergence within the family to indicate functional autonomy. The liberal use of putative splice junctions in the C-termini of SRC members gives rise to considerable sequence complexity between each member in these regions. For example, SRC-1 and TIF2/hSRC-2 are distinguished by a 65-amino acid deletion in TIF2/hSRC-2 with respect to SRC-1 (Fig. 2). Such structural anomalies between the members of the SRC family are reflected by differences both in their immunoreactivity and in their functional characteristics. Microinjection into cells of anti-NCoA-1/mSRC-1 antibodies, but not anti-NCoA-2/mSRC-2 antibodies, prevented RAR-dependent transactivation of a retinoic acid response element (RARE)-linked reporter gene. Coinjection of NCoA-1/mSRC-1, NCoA-2/mSRC-2, or p/CIP/mSRC-3 expression vectors showed, however, that either NCoA-1/mSRC-1 *or* NCoA-2/mSRC-2, but not p/CIP/mSRC-3, could rescue transactivation of this reporter gene (50). This result correlates with the compensatory overexpression of GRIP1/mTIF2 (SRC-2) in the SRC-1 null mutant. and indicates the functional distinction between the SRC-1/SRC-2 and SRC-3 subfamilies. In addition, immunodepletion with anti-NCoA-1/mSRC-1 antibodies had no effect on cAMP- or interferon-α dependent reporters, indicating the dispensability of NCoA-1/mSRC-1 in classic CBP-mediated signaling pathways. While the functional importance of the sequence variations between hSRC-3 isoforms and p/CIP/mSRC-3 is unclear, p/CIP, unlike the hSRC-3 isoforms, preferentially enhanced interferon-α stimulation of a reporter gene, suggesting a closer functional similarity of p/CIP/mSRC-3 to CBP than the hSRC-3 isoforms. Furthermore, while p/CIP/mSRC-3 failed to significantly enhance RAR function (50), the hSRC-3 isoforms ACTR (53), RAC3 (54), and TRAM-1 (56) markedly enhance transactivation by RAR/RXR. These conflicting results are quite possibly a consequence of the C-terminal anomalies between p/CIP/mSRC-3 and the hSRC-3 isoforms. Comparison of the sequences of SRC-3 members indicates that they are encoded by the same gene in different species and are distinguishable by the length of their polyglutamine tract and the presence of a lengthy unrelated C-terminal sequence present only in the p/CIP/mSRC-3 isoform (Fig. 2).

Another piece of evidence indicating a degree of autonomy of the SRC-3 subfamily is the overexpression of AIB-1/hSRC-3 in primary breast tumors (55) against a background of relatively low expression levels of SRC-1 and TIF2/SRC-2. These results indicate that overexpression of AIB-1/hSRC-3 is a factor in the genesis and/or progression of these tumors, and the stimulus for growth that it may afford is not limited by the comparatively low levels in these tumors of SRC-1 and TIF2/mSRC-2. In their totality, the structural discrepancies between SRC family members indicate functional diversity that may determine their interaction with nuclear receptors, with other promoter-specific transcription factors, and with other transcriptional coregulators.

e. The LXXLL/NR box motif. Detailed scrutiny of the receptor-interacting domains of RIP-140 (*Section II.B.1*) and SRC family members (50, 60) identified a conserved motif, LXXLL (where L is leucine, X is any amino acid), termed the nuclear receptor (NR) box (Ref. 61; Fig. 2), which is necessary and sufficient to mediate binding of the coactivators to liganded nuclear receptors. Three such motifs are conserved in SRC family members, and an additional NR box is present in the extreme C terminus of h/m (human/mouse) SRC-1 (Fig. 2). Secondary structure analysis of these motifs has indicated that they form amphipathic α-helices and that the conserved leucines form a hydrophobic surface on one face of the helix. The role of the NR box in mediating ligand-dependent receptor-coactivator interactions is signified by its conservation in the central portions of all three SRC family members (Fig. 2), to which domains mediating interactions with nuclear receptors have been localized (33, 50, 51, 54). Furthermore, the nonconserved NR box motif of h/mSRC-1 is present in its C terminus, which mediates the hormone-dependent interaction of hSRC-1 with PR (33). The mutation of key residues in the four NR boxes of hSRC-1, (I–III in the central portion of the protein and IV in the extreme carboxyl terminus) has been shown to abolish interaction with AF-2 of the ER but does not affect the interaction of hSRC-1 with CBP. In addition, this hSRC-1 mutant fails to coactivate the ligand-dependent activity of ER (60). In a broader context, the importance of the NR box motif is indicated by its presence in a wide variety of nuclear receptor coregulators, including E3 ubiqutin-protein ligases (*Section II.B.3.a*), TRAPs (*Section II.B.3.e*), p300/CBP (*Section II.B.5*), and TIF-1s (*Section IV.D.2*). A detailed approach to the question of the significance of multiple NR boxes in receptor-coactivator interactions (61) suggests that distinct NR box motifs exhibit differential binding to different receptors. It has become apparent that sequence anomalies around individual NR boxes might determine their binding affinity for the AF-2 ligand-induced hydrophobic groove of nuclear receptors (62). Indeed, the notion that the LXXLL motif is an immutable requirement for interaction with receptor LBDs has been challenged by the ability of the FXXLL motif of NSD-1 (nuclear receptor-binding SET domain-containing protein 1, *Section IV.D.3*) to mediate its interaction with nuclear receptor LBDs (63).

Recent studies have shed light on the series of events that accompany ligand interpretation and coactivator interaction

with the AF-2 of nuclear receptors. Feng *et al.* (64) have dissected the interaction between the TR AF-2 and GRIP1/mSRC-2 and have described the appearance of a hydrophobic groove in the ligand-bound AF-2 of TR (and ER), the interactive surface of which is highly conserved. A peptide modeled upon a GRIP1/mSRC-2 NR box recognizes a hydrophobic groove in the TR LBD lined by a series of residues, the deletion of any of which abrogates GRIP1/mSRC-2 peptide binding and TR transactivation (64). The critical role in AF-2 activity of an agonist-induced conformational change in the region of helix 12 of nuclear receptors has been well documented (65). By presenting crystallographic evidence that implicates helix 12 of tamoxifen-bound ER as a steric impediment to the binding of GRIP1/mSRC-2 to the ER, Shiau *et al.* (66) have shed light on the differential affinity of agonist and antagonist-bound receptor for coactivator. As is the case with TR (64), the NR box peptide occupies a hydrophobic groove fashioned by helices 3, 4, 5, and 12 of ligand-bound ER. Conversely, antagonist-induced apposition of helix 12 to the hydrophobic groove does not form part of an interactive surface, but rather occludes residues critical for the interaction between ER and the NR box peptide (66). The 2.2 Å resolution crystal structure of the ligand-bound PPARγ-SRC-1 complex (67) has highlighted the role of a "charge clamp" of conserved glutamate and lysine residues in the PPARγ-LBD that make contact with backbone atoms of the NR boxes of SRC-1. In addition, tandem NR boxes of the SRC-1 moiety were shown to contact with both members of a PPARγ homodimer, hinting at a possible further role of multiple NR box motifs in coregulators.

3. Other coactivators.

a. E3 ubiquitin-protein ligases: E6-AP and RPF-1. Using a yeast two-hybrid screen with the hPR as a bait, our laboratory has recently identified a PR-interacting protein that is identical to the E6 papillomavirus-associated protein E6-AP (68). E6-AP, an E3 ubiquitin-proteins ligase that targets proteins for degradation by the ubiquitin pathway, interacts with and coactivates hormone-dependent transactivation by members of the nuclear receptor superfamily. Further supporting its identity as a coactivator, E6-AP reverses squelching between ER and PR and contains an intrinsic activation function in its N-terminal domain. Tandem NR boxes (*Section II.B.2.e*) are present in its C-terminal receptor-interacting region. E6-AP was originally identified through its association with the papillomavirus E6 protein: a complex of E6 and E6-AP was shown to target the p53 tumor-suppressor protein for degradation (69). Interestingly, however, the ubiquitin ligase activity of E6-AP is separable from its coactivation function. E6-AP is closely related to the E3 ubiquitin-protein ligase RPF-1, the human homolog of yeast RSP-5, a protein shown to enhance PR and GR transactivation in mammalian cells (70). Our laboratory has recently shown that E6-AP and RPF-1 synergistically enhance PR transactivation in mammalian cells. In addition, these proteins copurify by gel filtration, indicating that their synergistic coactivation of PR might be related to their presence in a common complex (58).

b. L7/SPA. A two-hybrid screen of a HeLa cDNA library using the PR antagonist RU486-bound PR D/E domain as a bait isolated a 27-kDa protein, L7/SPA, a previously described nuclear protein having no known function (71). L7/SPA increases the partial agonist activity of 4-HT-occupied ER and RU486-occupied PR or GR by 3- to 10-fold *in vitro* but does not influence the activity of the agonist-bound receptor. Interestingly, the antihormonal effects of the pure antiestrogen ICI164384 on ER and the pure antiprogestin ZK98299 on PR could not be offset by coexpression of L7/SPA.

c. TLS. Powers *et al.* (72) used murine RXR to isolate a 65-kDa protein, termed translocated in liposarcoma (TLS), a protein previously identified as a member of the RNP family of nuclear RNA binding proteins. Translocation-induced fusion of this protein to a DNA-binding protein, CHOP, had been previously shown to result in a potent chimeric transactivator. High-affinity binding of TLS to DNA-bound TR was demonstrated, as was the interaction of TLS with TR *in vivo*. It was suggested that TLS may enhance receptor protein or RNA stability, but this is yet to be determined. Intriguingly, TLS bears significant sequence similarity to $hTAF_{II}68$, a TFIID/RNA Pol II-associated protein (73).

d. Trip-1/Sug-1. Lee *et al.* (74) have identified a protein, Trip-1, that interacts with TR and RXR baits in a yeast two-hybrid assay in a ligand-dependent manner. It was identified as a member of the CAD (conserved ATPase domains) family of proteins and exhibits significant sequence identity with the yeast transcriptional coregulator, Sug1, originally identified as a suppressor of a mutation in the transcriptional activation domain of the yeast activator Gal4. Although Sug1 was originally postulated to be a component of the RNA Pol II holoenzyme complex, Rubin *et al.* (75) have reported its copurification with the 2MDa yeast 26 proteosome complex and have correlated this with reduced ubiquitin-dependent proteolysis in *sug1* mutants. Along with the identification of the E3 ubiquitin-ligases, E6-AP and RPF-1, as coactivators of PR transactivation, these results reiterate the importance of protein degradation pathways in receptor action, although the exact role of such pathways is unknown. Trip-1 does not contain consensus LXXLL/NR box motifs (*Section II.B.2.e*), evidence of the existence of binding determinants, other than NR boxes, which govern interactions between nuclear receptors and their coactivators.

e. TRAPs/DRIPs. Recent biochemical approaches have permitted the identification and extensive characterization of multiprotein complexes that interact with liganded nuclear receptors. Fondell *et al.* (76) employed one such biochemical purification strategy to isolate TR-associated proteins. They showed that epitope-tagged TR purified from HeLa cells cultured in the presence of thyroid hormone was associated with a group of distinct nuclear proteins termed TRAPs (TR-associated proteins). Supplementation of an *in vitro* transcription system with the TR/TRAP complex enhanced the transcriptional activity of a promoter driven by thyroid hormone response elements on naked, chromatin-free DNA (76). While the TRAPs were shown initially to be immunologically distinct from SRCs (*Section II.B.2*), CBP (*Section II.B.5*), TIF-1s (*Section IV.D.2*), RIP140 (*Section II.B.1*), and TAF_{II}s (*Section II.B.3.g*), it has since been demonstrated that a 220-kDa member of the complex, TRAP 220, is identical to the PPARγ-binding protein, PBP (*Section II.B.3.h*; Ref. 77). Adopting a similar approach, Freedman and colleagues have presented similar data with respect to the VDR (78). Purified VDR

recruited a complex of proteins (DRIPs or VDR-interacting proteins) that is homologous to the TRAP complex. The DRIPs, which range in size from 70–230 kDa, were shown not to contain SRC family members, p300/CBP, or other characterized coactivators. As with TRAPs for TR, DRIPs were shown to modestly enhance the activity of VDR in a cell-free ligand-dependent transcription assay (78).

The role of such morphologically distinct complexes in receptor activation is currently unclear, but a model has been proposed in which these complexes might assume significant roles in repetitive rounds of transcription mediated by TR and VDR. In such a scenario, initial recruitment of chromatin-modifying complexes containing the cointegrators p300/CBP (*Section II.B.5*) and members of the SRC family (*Section II.B.2*), would be followed by displacement of some of these complexes and interaction of receptor with TRAP/DRIP-like complexes to form a link with general initiation factors (*Section I.B*; Ref. 79). While support for such a model arises from the identification of TRAP/DRIP components in a complex, SMCC, containing human homologs of yeast mediator/RNA Pol II holoenzyme factors (80), it should be noted that CBP itself exists in a stable complex with RNA Pol II (81). An alternative model arises from the interesting observation that in the DRIP study, ER-LBD failed to appreciably recruit DRIPs (78), raising the possibility that TRAPs/DRIPs represent a type II receptor-specific complex.

f. Positive cofactors (PCs). Two positive cofactors, PC2 and PC4, derived from the upstream stimulatory activity (USA) cofactor fraction, act synergistically to mediate thyroid hormone-dependent activation either by TR or by a TR-TRAP complex in a reconstituted *in vitro* system comprised of purified factors and naked DNA templates (82). PC4 is a general coactivator that functions cooperatively with TAF_{II}s and mediates functional interactions between enhancer-bound activators and the general transcription machinery of RNA Pol II-transcribed genes (83) and is related to viral immediate-early transcriptional regulators (84). In the absence of TAF_{II}s and TFIIH, PC4 strongly represses transcription initiation, while simultaneously promoting the formation of preinitiation complexes. Upon concerted phosphorylation by TFIIH and distinct TAF_{II}s (*e.g.*, $TAF_{II}250$), PC4 elicits full coactivator potential, indicating a situation reminiscent of that reported for activated transcription at prokaryotic ρ-dependent promoters.

g. TAF_{II}s. The specific functional interaction of the AF-2 of different nuclear receptors with distinct TAF_{II}s has been well documented, and these interactions may serve to determine the specificity of the transcriptional response at a promoter. Sequestration of $TAF_{II}30$ by ER has been shown to be necessary for ligand-dependent activation by the ER (10). In addition, $TAF_{II}28$ selectively coactivates the AF-2 of RXR, an effect not observed for ER or VDR (85). To corroborate a specific role of TAF_{II}s in receptor transactivation, Mengus *et al.* (86) have demonstrated the ability of $TAF_{II}135$ to coactivate RAR, VDR, and TR, but not RXR or ER, and have speculated that $TAF_{II}135$ might enhance recruitment of TFIID by nuclear receptor AF-2s. Viewed in their entirety, these observations indicate that recruitment of distinct TFIID complexes at diverse promoters might be a component of nuclear receptor action.

h. PBP/TRAP220/TRIP2/mPIP9. The PPAR members of the nuclear receptor superfamily regulate the expression of genes involved in lipid metabolism and adipocyte differentiation. A recent study (77) has shown that a 165-kDa PPARγ-binding protein, PBP, binds to and enhances the transcriptional activity of PPARγ. PBP has exact sequence similarity with TRAP220/TRIP2/mPIP9 (79, 87, 88) and has a broad binding specificity for type II receptors, including RARα, RXR, and TRβ1. Furthermore, PBP contains two LXXLL motifs and is widely expressed in adult mice tissues. Given the limited binding specificity of PBP/TRAP220/TRIP2/mPIP9, the possibility exists that it represents a type II receptor-specific coactivator, although this has yet to be demonstrated on a functional level.

i. NCoA-62. Baudino *et al.* (89) used a yeast two-hybrid screen with VDR, RAR, and RXR to isolate a coactivator, NCoA-62, which manifested a broad specificity in both its interaction with, and coactivation of, nuclear receptors. NCoA-62 strongly coactivated VDR-mediated transcriptional activation, but more modestly enhanced ligand-dependent transcription from minimal promoters controlled by RAR, ER, and GR. While NCoA-62 lacks perfect LXXLL motifs, close inspection of its sequence indicates the presence of the pentapeptides LXXFL and LXXAL. The hydrophobic character of these peptides resembles that of LXXML, a motif involved in the intramolecular contact of helix 12 with helix 3 and helix 5 in raloxifene- and tamoxifen-bound ER (see *Section II.B.2.e*), interactions thought to act as a steric impediment to NR-box binding (66). These data, while circumstantial, indicate that the imperfect NCoA-62 motifs might suffice to mediate its interaction with the AF-2 region of its nuclear receptor-binding partners.

j. TSC-2 (tuberous sclerosis-2). Tuberous sclerosis is an autosomal dominant disorder characterized by the appearance of benign tumors in a wide variety of tissues, including the eye, kidney, heart, and brain, where they cause epilepsy and mental retardation (90). TSC has been genetically linked to two loci in humans, one located on chromosome 16p13 (91), and the TSC-2 gene product has been characterized as a 200-kDa protein containing a short N-terminal leucine zipper and a C-terminal region homologous to the RAP1 GTPase-activating protein. In a yeast two-hybrid screen using RXR as a bait, Henry *et al.* (92) isolated a gene bearing 98% homology to that encoding TSC-2, and TSC-2 was also shown to interact with RXR in an *in vitro* pull-down assay. In transient transfection assays, TSC-2 was shown to stimulate PPARγ and VDR-mediated transactivation. A mechanism for TSC-2 in nuclear transport and/or cytoplasmic signaling was suggested (92), but its role in nuclear receptor transactivation is yet to be clearly established.

4. Selective coactivators.

a. SRA. Our laboratory has recently isolated and functionally characterized a novel transcriptional coactivator, termed steroid receptor RNA activator, or SRA (93). SRA was originally isolated in a yeast two-hybrid screen using the amino-terminal domain of PR-A. When overexpressed in mammalian cells, recombinant SRA specifically enhances endogenous steroid receptor AF-1-mediated transactivation by 5- to 10-fold without altering the level of

basal transcription. Several pieces of evidence indicate that SRA functions not as a protein but as an RNA transcript, introducing an entirely novel concept not only in nuclear receptor action, but in eukaryotic transcription as a whole. Transactivation analysis of multiple SRA frameshift and stop codon-containing mutants indicates that these mutants retain the capacity to coactivate steroid receptors. Further evidence of the identity of SRA has been provided by transfection experiments in the presence of the *de novo* protein synthesis inhibitor cycloheximide, in which SRA retained its ability to coactivate a reporter gene, whereas protein coregulators such as hSRC-1 and CBP did not. In addition, biochemical analysis has suggested that the SRA transcript is present in an hSRC-1 complex that is recruited by steroid receptors *in vivo*. We have shown that SRA is expressed in a tissue-specific manner, *e.g.*, in brain, where it colocalizes with the expression of certain steroid receptors. Given its evident functional selectivity, we have proposed a model in which SRA, as an AF-1 coactivator, functions to confer specificity upon coactivator complexes to specifically enhance steroid receptor-mediated transcription (93).

b. ARAs. ARA70 (androgen receptor activator-70), a 70-kDa human protein isolated on the basis of ligand-dependent interaction with an AR AF-2 bait in a yeast two-hybrid screen, was reported to enhance AR transactivation in DU145 human prostate cells, but had no effect on transactivation by other nuclear receptors (94). Unlike the SRC family members hSRC-1 and TIF2/hSRC-2, ARA70 has been shown to be capable of enhancing the partial agonist activity of hormonal antagonists (95).

c. Trip230. Like AIB-1/hSRC-3 (*Section II.B.2.c*), the thyroid receptor coactivator Trip230 highlights the potential role of coactivators in disease states (96). Trip230 was isolated as a partner of the Rb gene product, the interaction being mediated by the N terminus of the Rb protein. 14q31, The chromosomal locale of the Trip230 gene, is a locus to which several abnormalities of thyroid hormone response, including Graves' disease and congenital hyperthyroidism, have been linked, implicating Trip230 as a factor involved in the thyroid hormone response. While Trip230 binds TR in a thyroid hormone-dependent manner and enhances TR-dependent transactivation, thyroid hormone has no effect on its interaction with Rb. Coexpression of Rb abolishes the enhancement of TR transactivation effected by Trip230, indicating a functional antagonism between Rb- and TR-mediated pathways.

d. PGC-1. Puigserver *et al.* (97) have identified a novel coactivator, PGC-1 (PPARγ coactivator-1), which is preferentially expressed in brown fat and skeletal muscle and which enhances transactivation by TR and PPARγ on the uncoupling promoter-1 (UCP-1). In contrast to AF-2 coactivators, PGC-1 was shown to bind preferentially to a region outside the AF-2 domain of PPARγ-1, in the hinge (D) region. Overexpression of PGC-1 in white adipose tissue activates UCP-1 and key mitochondrial enzymes. In addition, exposure to low temperatures enhances expression of the PGC-1 gene, and this has been suggested to be a key mechanism underlying adaptive thermogenesis in mammals. PGC-1 is a striking example of the control over coactivator function exerted by environmental stimuli and is an intriguing insight into the mechanism whereby selective regulation of coactivator expression mediates a specific and isolated transcriptional response *in vivo*.

e. HMGs. The HMG-1 (high-mobility group) and HMG-2 proteins occupy a unique niche among characterized nuclear coactivators by selectively enhancing the DNA-binding activity of the type I steroid receptor subfamily (98). Transient transfection assays showed that cotransfection of HMGs with different steroid receptors resulted in enhancement of PR, GR, and AR transactivation, but not that of VDR. While HMG-1 and -2 interacted only transiently with purified PR in solution, and had no affinity for PRE (progesterone response element) *per se*, PRE binding by PR resulted in the formation of a stable PR-HMG-PRE complex. These results suggested that DNA binding by PR is concomitant with its interaction with HMGs, which serve to stabilize the association of PR with its response element.

5. Cointegrators: CBP/p300. CREB-binding protein (CBP) was initially characterized as a coactivator required for efficient activation of cAMP-regulated promoters by the transcriptional activator cAMP-response element-binding protein (CREB, Ref. 99). Several studies implicate CBP as a coactivator of multiple transcriptional activators, including p53 (100), NF-κB (101), and nuclear receptors (45, 102, 103). In addition, direct interactions between CBP and RXR, TR, and ER are mediated by the N-terminal domain of CBP (45), which contains an NR-box indispensable for receptor interaction (60).

In addition to its interactions with nuclear receptors, CBP interacts with members of the SRC family, including mSRC-1/NCoA-1 (45), TIF2/hSRC-2 (51), and p/CIP/mSRC-3 (50), indicating that it may form a ternary complex with SRC family members and nuclear receptors. Functional evidence suggests such a complex may exist, since CBP synergizes with hSRC-1 in the transactivation of ER and PR transactivation (104). Biochemical evidence suggests, however, that CBP does not form a stable complex with hSRC-1 (58), and it has been shown that the interactions of liganded ER (105) and PR (58) with CBP are relatively weak in comparison with the recruitment by these receptors of hSRC-1-containing complexes. We have proposed (58) that an initial receptor/hSRC-1 complex recruits other functionally diverse complexes containing coactivators such as CBP. In support of this, CBP is ineffective in restoring activity to an RARE-linked reporter gene after immunodepletion of NCoA-1/mSRC-1 (50), suggesting that CBP might require SRC-1 complexes as a platform to effect its coactivation of nuclear receptors. An overall model of CBP action (45) suggests that, as a common limiting cofactor for diverse transcriptional activators and coactivators, it acts as a cellular cointegrator to collate multiple afferent signals into an integrated response at promoters containing multiple *cis*-acting elements. A critical physiological role of CBP is indicated by the fact that Rubinstein-Taybi syndrome, a rare disorder characterized by mental retardation and numerous physical deformities, is associated with mutation of *CBP* in humans (106).

p300 (107) Shares many of the functional properties of CBP, including transcriptional enhancement of diverse tran-

scription factors such as MyoD (108), p53 (100), and nuclear receptors (102). In addition, p300 associates with mSRC-1 (109) and interacts with ER in a ligand-dependent manner (110). This functional redundancy is not complete however: targeted deletion of the p300 locus (111) indicates that functional CBP in such animals is insufficient to prevent defects due to loss of p300 in neurulation, cell proliferation, and heart development, as well as embryonic lethality. Kraus and Kadonaga (112), observing that p300 and ER synergistically activate cell-free transcription in the presence of chromatin, have postulated a cooperative "fire and reload" mechanism, in which p300 and ER cooperatively enhance transcription during a single round of transcription ("fire"), but only ER is required for reassembly of the transcriptional preinitiation complex ("reload"). Their results highlight the functional distinction between different events at a transcriptionally active promoter and the individual role of diverse factors in the fluid and intricate process of transcriptional activation.

III. Nuclear Receptor Corepressors

A. Background

1. Repression of basal transcription by nuclear receptors. Transcriptional repression, or silencing, refers to the ability of type II receptors to lower basal promoter activity in the absence of ligand. A number of different mechanisms have been proposed for silencing by nuclear receptors. Passive repression refers to competition either for DNA binding or for dimerization partners, both mechanisms resulting in steric hindrance by the unliganded receptor on the promoter, blocking access of activators or basal factors (113). In addition, inactive heterodimer formation with other type II receptors has been documented for TR (114, 115). Conversely, in the active silencing model, unliganded receptor either directly affects transcription initiation, or recruits an array of factors, the function of which is to create an environment that is incompatible with proper assembly of a preinitiation complex, a mechanism also known as transrepression. Data supporting direct contacts between nuclear receptors and general transcription factors that result in transcriptional silencing are limited. Baniahmad *et al.* (116) sketched a possible mechanism for silencing by TR, envisaging sequestration of the basal transcription factor TFIIB by domains in the C terminus of the unliganded TR. Maximal repression by TR, however, was contingent upon other more N-terminal domains that did not interact with TFIIB.

2. Evidence for the existence of corepressors. In contrast to cellular TR, its viral counterpart, the oncogene product v-*erb*A fails to bind hormone and is a constitutive repressor of transcription of thyroid hormone-responsive genes (117). Baniahmad *et al.* (118) demonstrated the existence of active silencing domains in TR and showed that these domains functioned as repressors when fused to a heterologous DBD. In experiments symmetrical to those that implied the existence of nuclear receptor coactivators (*Section II.A*), our laboratory showed that the silencing activity of TR could be greatly reduced (squelched) in transient cotransfection assays by coexpression of either the C terminus of v-*erb*A or the unliganded TR-LBD. Such interference predicted the existence of soluble corepressors for TR and other type II receptors, present in limiting cellular concentrations (119, 120). Table 2 summarizes the functional properties of characterized nuclear receptor corepressors.

B. Corepressors

1. NCoR/RIP-13. Biochemical studies of cellular proteins associated with unliganded TR and RAR have identified a 270-kDa protein termed NCoR (nuclear receptor corepressor; Ref. 121), also isolated as RIP-13 (122). While Horlein *et al.* (121) reported that NCoR was specifically recruited by unliganded TRα and RARα, and that little or no interaction was observed between NCoR and RXR, VDR, ER, or GR, Seol *et al.* (122) isolated RIP13 using RXR as a bait. Mutational analysis of the TR LBD has identified a domain, termed the NCoR box, which is indispensable for the interaction of receptor and NCoR. Loss of the NCoR box attenuates repression by the unliganded TRα, strongly suggesting that interaction

TABLE 2. Nuclear receptor corepressors

Factor	Alternative designations	Related proteins	Comments	References
NCoR	RIP-13	SMRT	Interacts with and corepresses unliganded TRα, RARα & COUP-TF1, RevErb and DAX-1; contains autonomous repression domains; reduces RU486/PR partial agonist activity; specifically degraded by 26S proteosome after binding Siah; associates with PML-RARα fusion proteins in acute promyelocytic anemia.	71, 121–127, 131, 133, 134, 138, 140–142, 144, 149–151, 206, 207
SMRT	TRAC2	NCoR	Interacts with and corepresses unliganded TR and RAR; significant sequence similarity with NCoR; contains an autonomous repression domains; reduces tamoxifen/ER and RU486/PR partial agonist activity; associates with PML-RAR fusion proteins in acute promyelocytic anemia.	71, 125, 128–142, 148, 150, 151, 208
TRUP	SURF-3, PLA-X, L7a		Reduces transactivation by TR and RAR; decreases DNA binding by RXR heterodimers.	143
SUNCoR			Contains autonomous repression domain; corepresses TR and RevErb.	144
NURD			Couples ATPase activity to histone deacetylation; immunodepletion decreases repression by TR; contains NCoR-related subunit.	212

with NCoR is required for efficient TRα and RARα-mediated transcriptional repression (121). As is the case with several nuclear receptor coactivators, the existence of NCoR isoforms has been postulated based upon the isolation of cDNAs encoding putative splice variants, although no direct evidence of their existence has been obtained.

To substantiate the identification of NCoR as a mediator of ligand-independent repression, fusion of NCoR to the Gal4DBD effects potent repression at a promoter bearing Gal4DBD-binding sites (121). Deletion mutations of NCoR have identified two receptor-interacting domains (RIDs) in the C-terminal portion of the protein that are required for nuclear receptor interaction (122). Further N-terminal in the NCoR molecule are three repression domains, one at the extreme N terminus (RI) and two more centrally located (RII and RIII), to which the intrinsic repressive functions characteristic of NCoR have been ascribed. Analogous to the dominant negative activity of the C-terminal receptor-interacting domain of the coactivator hSRC-1 (*Section II.B.2.a*), coexpression of the RIDs-bearing domain of NCoR abolishes repression effected by unliganded TR and RAR (123). More recently, studies in our laboratory and others have indicated the role of NCoR in mediating the transcriptional silencing properties of members of the orphan receptor subfamily, including Rev-Erb (124), chicken ovalbumin upstream promoter transcription factors (COUP-TFs; Ref. 125) and DAX-1 (126). Moreover, Muscat *et al.* (127) showed the ability of NCoR and its variants RIP-13α and RIP-13δ1 to directly interact with TFIIB, $TAF_{II}32$, and $TAF_{II}70$, indicating that corepressors may function, at least in part, by mediating repressive interactions of unliganded receptors with components of the basal transcription apparatus (transrepression).

2. *SMRT/TRAC2.* SMRT (silencing mediator for retinoid and thyroid hormone receptor) was isolated by a yeast two-hybrid screen of a human lymphocyte cDNA library using RXR as a bait (128). SMRT was also identified as TRAC2 (T_3 receptor-associating cofactor 2), a protein isolated on the basis of its interaction with RAR, RXR, and TR (129). While significant sequence similarity exists between the N- and C termini of SMRT and NCoR (130), the N terminus of NCoR contains two repressor domains that are not present in SMRT. RAR and TR interact strongly with SMRT and RXR in a far-Western analysis, and addition of ligand to these receptors induces dissociation from SMRT, but not from RXR. Furthermore, in a yeast two-hybrid assay, a strong ligand-reversible interaction with SMRT has been observed for the LBDs of TR and RAR (128). In addition, direct recruitment of SMRT to a promoter by fusion with a heterologous DBD results in substantial repression of the basal promoter activity (128). SMRT/TRAC2 contains two C-terminal receptor-interacting domains, RID-1 and RID-2, which, analogous to the selective recruitment of receptors by distinct NR boxes (*Section II.B.2.e*), interact differently with individual receptors. RARα, for example, binds RID-1 exclusively, whereas TR binds both domains with equal affinity (131). Sande and Privalsky (129) have described the ability of an amino-terminal truncation of SMRT/TRAC2, named TRAC1, to act as a dominant-negative inhibitor of TRAC2, but the biological significance of this is yet to be determined.

SMRT reverses the squelching of Gal4DBD-RAR silencing by RAR 403, a RAR mutant lacking the RAR C terminus and a robust repressor of the basal activity of RARE-containing promoters (132). In contrast to the ligand-reversible association of full-length RAR and RAR-LBD with SMRT, RAR 403 retains the interaction with SMRT in the presence of ligand. Similarly, the association of the constitutively silencing TR-derived oncogene product v-*erb*A with SMRT is unaffected by ligand, and ectopic expression of SMRT reverses the squelching of Gal4DBD-TR silencing by overexpressed v-*erb*A. The physiological significance of these results has been illuminated somewhat by Yoh *et al.* (133), who have demonstrated that a variety of mutations in the TRα gene, which are associated with general resistance to thyroid hormone, result in strong constitutive retention of the corepressors SMRT and NCoR by the mutant receptors.

The inability of SMRT to interact with constitutively activating TR mutants further hints at its role as a transcriptional corepressor. A TR Pro→Arg mutant, TR-160, devoid of silencing activity but capable of hormone-dependent transactivation, shows little affinity for SMRT in an *in vitro* pull-down assay (128). In addition, a TR-LBD mutant that does not silence but retains its transactivation function, does not interact with the C-terminal RID domain of SMRT (125). Tagami *et al.* (134) have shown that both NCoR and SMRT are capable of functioning as transcriptional activators at negative thyroid response elements (TREs), suggesting that the repressive properties of these corepressors are not intrinsic. Genes regulated by negative TREs are stimulated by unliganded TR and repressed upon the addition of thyroid hormone. In this study, ectopic expression of NCoR and SMRT enhanced basal transcription of a negative TRE in a hormone-dependent manner, whereas a TR mutant, which failed to interact with NCoR, did not activate transcription in this assay (134). These and other results (127) suggest that specific *cis*-acting factors can modulate the function of corepressors and that corepressors may mediate productive, as well as repressive, interactions with general transcription factors.

3. *NCoR and SMRT: functional similarities and divergence.* NCoR and SMRT appear to be less than discriminate in their binding of repressive transcription factors, suggesting they may have a more general role in transcriptional repression than was initially considered. Dhordain *et al.* (135) have described the interaction of the POZ motif of the non-Hodgkin's lymphoma-associated protein LAZ3/BCL6 with SMRT. The promiscuous interaction of SMRT and NCoR with POZ motif-containing proteins is further illustrated by certain cases of acute promyelocytic leukemia, a disease characterized by incomplete leukocytic differentiation and appearance of leukemic blast cells. Novel fusions of the RARα gene have been identified (136–138) that arise from chromosomal translocations with loci containing the genes encoding the PML (promyelocytic leukemia) and PLZF (promyelocytic leukemia zinc finger) proteins. The resultant proteins, PML-RARα and PLZF-RARα, were shown to retain the RARα DBD and LBD. Clinically, PML-RARα patients achieve complete remission upon administration of pharmacological doses of all-*trans* RA. PLZF-RARα patients, con-

versely, respond poorly to such therapeutic intervention. These phenomena were directly attributed to the constitutive recruitment of NCoR and SMRT by the PLZF protein, an interaction not subject to regulation by binding of ligand by the RARα moiety. The PML-RARα fusion, however, binds NCoR and SMRT only through the RARα LBD, explaining the ability of such patients to eventually respond to RA. To further illustrate its promiscuity, SMRT also interacts with CBF-1/RBP-Jκ, the mammalian homolog of the *Drosophila suppressor of hairless*, which switches from a transcriptional repressor to an activator upon binding of the ligand *notch*. In the absence of ligand, CBF-1/RBP-Jκ is part of a repressor complex containing SMRT, which subsequently dissociates when *notch* binds (139).

While NCoR and SMRT are structurally similar (121, 128), they differ functionally in several respects. The molecular basis of heterogeneity of function among RAR isoforms has been ascribed to their differential interaction with SMRT and NCoR: whereas RARα and RARγ interact with both corepressors, RARβ exhibits no affinity for either SMRT or NCoR (131). The work of Baniahmad *et al.* (140) indicates that the weak repression of basal transcription by TR in CV-1 cells can be amplified by ectopic expression of SMRT, but not NCoR. These corepressors differ most notably, however, in the mediation of transcriptional repression by certain orphan receptors. Crawford *et al.* (126) showed that the orphan receptor DAX-1, which interacts with NCoR, does not recruit SMRT. Similarly, when bound to DNA, the orphan RevErb exclusively recruits NCoR, but does not require SMRT to effect transcriptional repression (141). Zhang *et al.* (142) demonstrated that repression by RevErb is cell line-specific, such that RevErb represses in 293T cells, but not in N18 neuroblastoma cells. They found that while the NCoR transcript is found in both cell types, NCoR protein is greatly reduced in the N18 cells. Yeast two-hybrid screening using the N-terminal portion of NCoR isolated a protein, present in N18 cells but absent in 293T cells, termed Siah (*Seven-in-absentia* homolog), a ring finger protein initially identified as a factor in *Drosophila sevenless* signaling. Siah is a potent mediator of NCoR down-regulation, decreasing the half-life of NCoR by approximately 5-fold. Siah-mediated down-regulation of NCoR has been linked to the 26S proteosome of the ubiquitin pathway: inhibition of the 26S proteosome prevents NCoR degradation and restores repression of RevErb (see also *Sections II.B.3.a* and *II.B.3.d*). Crucially, Siah does not interact with the N-terminal repression domain of SMRT and hence selectively targets NCoR for proteosomal degradation. Unlike RevErb repression, repression by TR is largely unaffected by endogenous Siah, consistent with its ability to recruit SMRT in addition to NCoR (142). The discriminate degradation of NCoR illustrates a mechanism whereby signaling by the function of one receptor type can be selectively abolished and highlights the multiple layers of control over nuclear receptor function.

4. *Other corepressors.*

a. TRUP/SURF-3/PLA-X. Burris *et al.* (143) have identified a protein, TRUP (thyroid receptor-uncoupling protein), which attenuates hormone-dependent transactivation by TR and RAR, but which has no effect on transactivation by ER or RXR. Sequence comparison of TRUP indicates its complete identity with the nuclear proteins SURF-3 and PLA-X. In addition, TRUP opposes ligand-dependent activation by TR in transient cotransfection. The ability of TRUP to diminish the hormone-dependent transactivation and silencing properties of TR has been attributed to the decreased ability of TR:RXR and RAR:RXR heterodimers to interact with their cognate hormone response elements (HREs). The capacity of TRUP to modulate receptor action in this manner represents another distinct mode of control among coregulators.

b. SUN-CoR. Zamir *et al.* (144) have isolated SUNCoR (small ubiquitous nuclear corepressor), a highly basic 16-kDa corepressor that shows no homology to either NCoR or SMRT. SUN-CoR contains an intrinsic repression domain and enhances silencing of basal transcription by TR and RevErb. The potential role of SUNCoR as an additional functional element in corepressor complexes is evinced by its interaction *in vivo* with NCoR.

5. *Steroid hormone receptor repression.* Steroid hormone receptors have little DNA-binding activity in the absence of hormone; indeed, steroid receptors, including PR and GR, are sequestered in ternary interactions with hsp90 and hsp70 (1). Recently, considerable effort has been devoted to discerning the mode of action *in vitro* of synthetic steroid hormone antagonists such as RU486 and 4-HT. These ligands induce receptor dimerization and DNA binding, but the resultant receptor dimer is ineffectual in stimulating transactivation. They act either as partial agonists or antagonists, in a manner contingent upon the tissue or promoter context. While the effects of these ligands have been attributed in part to their ability to disrupt interactions of receptor and coactivator (27, 29, 33, 66), recent evidence suggests that they may also induce active repression by nuclear receptors by promoting their association with transcriptional corepressors *in vitro*.

We have investigated the mode of action of RU486 as a PR antagonist and, by providing evidence for the involvement of a cellular corepressor in PR action, have introduced a novel concept in steroid hormone receptor action (145). Observing that PR and GR mutants lacking a short C-terminal portion of the receptor can be specifically activated by RU486 (146, 147), our group postulated the existence of an intrinsic repressor function in this domain that inhibited the transcriptional activity of the RU486-bound receptor. An amino acid sequence was defined in the C terminus of the PR that contained an intrinsic repressive function when fused to a heterologous DBD, indicating that this region interacted with a soluble corepressor (145). Mutations within this amino acid sequence in the full-length protein resulted in a PR that stimulated transcription in the presence of RU486. Competitive overexpression of the putative repressor domain activated the RU486-bound wild-type PR without affecting hormone-dependent transactivation, indicating titration of a cellular corepressor responsible for down-regulating the transcriptional activity of RU486-bound receptor.

A number of studies have since demonstrated the interaction of nuclear receptor corepressors with antagonist-bound steroid receptors. Smith *et al.* (148) demonstrated that SMRT abrogates the ability of mixed antiestrogen to activate transcription of an ER-dependent gene. Furthermore, *in vitro*

interaction assays have indicated an association between ER and SMRT in the presence of 4-HT. It was suggested that tissue-specific variations in corepressor expression might explain the ability of antagonists to evoke an agonist-like response in some tissues but not others. Intriguingly, it has also been demonstrated that SMRT interacts with ligand-bound ER, raising the possibility that corepressors modulate ligand-dependent activation by nuclear receptors (148). Additionally, RU486-bound PR functions as a transcriptional activator in the presence of unliganded TR or 4-HT-bound ER, but loses this ability in the presence of liganded TR or agonist-bound ER (149). In a yeast two-hybrid screen, Jackson *et al.* (71) have shown that NCoR interacted with antagonist-bound PR-LBD and that overexpression of NCoR and SMRT markedly suppressed RU486- and 4-HT-mediated partial agonist activity, an effect reversible, in the case of the PR, by overexpression of the PR LBD. Adding physiological significance to these data, Lavinsky *et al.* (150) have correlated decreased levels of NCoR with acquisition of hormone resistance in a mouse breast cancer model. Wagner *et al.* (151) demonstrated that NCoR and SMRT preferentially associate with antagonist-bound PR and that the partial agonist activity of RU486-bound PR is ablated by overexpression of NCoR and SMRT. In total, these results indicate that steroid receptors occupied by mixed agonists/antagonists such as RU486 or 4-HT are not intrinsically transcriptionally inactive, and that their transactivation functions may be masked by binding of corepressors (149). These observations point to the possible physiological role of mixed agonists/antagonists in steroid receptor action, and pose the question: could similar compounds exist in nature?

IV. Nuclear Receptors and Chromatin

A. Background

Eukaryotic chromosomes (herein referred to loosely as chromatin) are organized in the steady state into a regularly repeating protein DNA unit termed the nucleosome. The basic protein unit of the nucleosome is the histone, a small, highly basic, globular moiety. Solution of the low-resolution structure of the nucleosome core particle indicated a structure comprising a histone octamer, made up of two copies each of histones H2A, H2B, H3, and H4, around which was wrapped 1.7 turns of a left-handed DNA superhelix (152). Higher tiers of organization are thought to involve the assembly of nucleosomes into chromatin domains. The net effect of this arrangement is to create a thermodynamic barrier against the access of transcription factors to their DNA substrate. Recent higher resolution studies have established the fine structure of the nucleosome particle and have highlighted the role of the histone amino-terminal tails in making internucleosomal contacts (153). This arrangement has important consequences for our interpretation of the mode of action of two classes of molecules thought to regulate the access of transcription factors to their cognate DNA elements, namely acetylases (*Section IV.C*) and deacetylases (*Section IV.E*).

A multistep model has been envisaged for transcriptional activation by nuclear receptors (36, 154–156). Binding of the activated receptor to the enhancer region directs modification of the local chromatin structure into a transcriptionally permissive state (derepression), followed by recruitment of GTFs to form a preinitiation complex at the promoter (activation). This section reviews recent findings that substantiate this model, discussing the mechanisms by which coregulators, through intrinsic and recruited chromatin-modifying activities, are thought to manipulate chromatin and facilitate efficient transcriptional regulation by nuclear receptors. Covalent modification of nucleosomal structure is regulated by the diametrically opposed activities of histone acetylation, correlated with gene activation, and histone deacetylation, generally associated with gene repression (Fig. 3). In addition, recruitment of ATPase complexes that effect noncovalent modifications of chromatin domains appears to be important for transcriptional regulation by nuclear receptors.

B. The MMTV and TRβA promoters

The functional consequences of the organization of hormone-responsive promoters in higher order chromatin structures is best illustrated by the MMTV and TRβA gene promoters. These promoters attest to the intimate structural and functional association between the DNA template and the protein component of chromatin.

The long terminal repeat sequences of the MMTV promoter are organized into a series of six positioned nucleosomes (157), directed by the primary nucleotide sequence of the promoter. The array is such that the *cis*-acting elements of the MMTV promoter adopt precise translational and rotational settings on the surface of the histone octamer that favor nuclear receptor binding while precluding the binding of the transactivators NF-1 and Oct-1. Several studies have documented a two-step model of synergistic enhancement of the MMTV promoter by steroid hormones and the NF-1 and Oct-1 transactivators, and the intrinsic role of the nucleosomal structure in this model (Ref. 158 and references therein). After induction by progestins, a rearrangement of the phasing of the nucleosomes exposes the NF-1 and Oct-1 sites and

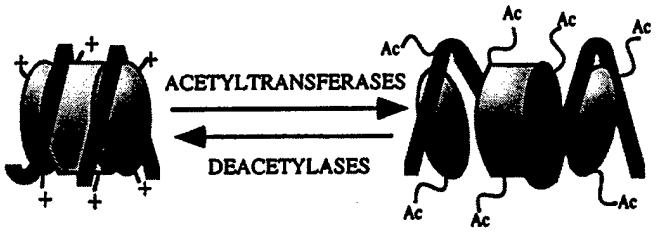

FIG. 3. Simplified model of the biochemical basis of nucleosomal remodeling by histone acetyltransferases and histone deacetylases. Catalytic transfer of acetyl groups to the terminal amino groups of lysine residues of histones H2A, H2B, H3, and H4 histones by histone acetylases (HATs) is thought to result in disruption of interactions between nucleosomes and DNA, between nucleosomes and neighboring nucleosomes, and possibly between nucleosomes and other proteins. The overall loss of compact nucleosomal structure facilitates access of transcriptional activators and coactivators to the promoter template. Conversely, recruitment of histone deacetylases (HDs) is thought to result in loss of the acetyl groups, reestablishing the coherence of the nucleosomal structure, and restricting access of transcription factors to the promoter. Other covalent modifications may also have a role in regulating nucleosome interactions.

primes the promoter for a rapid and synergistic response to these transcription factors.

Another promoter that exemplifies the influence of chromatin on events at hormone-inducible promoters is that of the *Xenopus* TRβA gene. Transcriptional initiation at this promoter is subject to autoregulation by thyroid hormone and TR (159). Wong *et al.* (156, 160) carried out a series of incisive experiments on the TRβA promoter using a *Xenopus* oocyte system, in which heterodimers of TR and RXR bound cognate HREs *in vivo* and were capable of alternately silencing or activating transcription in response to ligand. In the case of repression, simultaneous chromatin assembly and unliganded receptor heterodimer positioning were required for maximal transcriptional silencing, suggesting a synergistic role for chromatin in mediating silencing by the receptor. On the other hand, their results suggested that relief of this transcriptional repression by liganded TR comprises two distinct, independently regulated events: 1) extensive modifications of repressive chromatin structures, which are necessary but not sufficient to effect transcriptional initiation, and 2) interaction with GTFs resulting in assembly of a preinitiation complex (156, 160).

C. Coactivators and acetylation

Historically speaking, increased acetylation of histone tails has been correlated with transcriptional activity, whereas hypoacetylation has been associated with repression (161). The prevailing view has been that the major effect of the reduction of positive charge afforded by hyperacetylation of the amino-terminal histone tails is to uncouple their interaction with the negatively charged DNA, thereby creating an environment more accessible to transcription factors (Fig. 3). This theory has been modified somewhat by the recent solution of the nucleosome particles at 2.8 Å (153), which highlights nucleosomal-nucleosomal contacts made by the amino-terminal tails of histones, and suggests that an additional effect of selective acetylation of lysine residues in these tails may be to disrupt higher order chromatin structures (162).

Brownell *et al.* (163) identified histone acetyltransferase (HAT)-A, a *Tetrahymena* protein that contained acetyltransferase activity and showed close sequence similarity with the yeast transcriptional adaptor protein GCN5 (general control nonrepressed protein 5). Their discovery was the first indication that recruitment of histone acetylation activity by sequence-specific transcription factors might be involved in transcriptional regulation in eukaryotes. This was rapidly followed by the identification of the HAT activity of the general transcription factor $TAF_{II}250$ (164), implying a role for histone acetylation in access of TFIID to the promoter template. Initial indications of the role of acetylation of core histones in transcriptional regulation by nuclear receptors emerged from the identification of the intrinsic HAT activity of p300/CBP-associated factor (PCAF; 165), identified as a mammalian counterpart of yeast GCN5. Interestingly, GCN5 was characterized as a component of the yeast ADA complex, which is known to mediate AF-2-dependent activation by RXR and ER (166). PCAF interacts with p300 and CBP both by *in vitro* pull-down and by *in vivo* coimmunoprecipitation. The HAT activity of PCAF primarily targets histones H3 and H4 as substrates, exhibiting a preference for histone H3. PCAF interacts directly *in vitro* with p300/CBP (165), hSRC-1 (167), ACTR/hSRC-3 (53), and nuclear receptors (36, 168), interactions that may serve to stabilize a functional complex of receptor, SRC family members, PCAF, and p300/CBP on the promoter. Recent evidence suggests that PCAF exists in stable, preformed complexes with histone-like TAF_{II}s (169) in a manner akin to the arrangement of similar TAF_{II}s in the human GCN5 and yeast SAGA acetylase complexes (170). This striking finding raises the possibility of the evolutionary conservation of a mechanism whereby recruited GCN5/PCAF complexes assume the architectural role of local chromosomal histones during transcriptional activation.

HAT activity has also been identified as a property of the transcriptional cointegrators p300 and CBP (171, 172). Unlike PCAF, CBP and p300 can acetylate all four core histone types and, whereas CBP exhibits no substrate specificity, p300 HAT activity is directed primarily toward histone H3. HAT activity is also conserved in members of the SRC family, including ACTR/hSRC-3 (53) and hSRC-1 (167), although no such activity has been identified in TIF2/hSRC-2. The intrinsic histone acetylase activity of hSRC-1 maps to a carboxy-terminal region of SRC-1 and is specific for histones H3 and H4. Korzus *et al.* (173) have suggested that the apparent redundancy of HAT activity among nuclear receptor coregulators may be due to the requirement by diverse promoters of different combinations of HAT activities at different promoters. In support of this, hSRC-1 (58) and SRC-3 (N. J. McKenna, unpublished) complexes are biochemically distinct from those of CBP, p300, and PCAF, suggesting that combinatorial assembly by liganded receptor of these subcomplexes into larger complexes could occur in a cell- or promoter-specific manner (58).

While the discussion to this point has emphasized the well characterized role of cellular acetyltransferases in the catalytic acetylation of nucleosomal histones, it has become apparent recently that the spectrum of substrates for these enzymes extends to nonhistone proteins, implying a broader regulatory role for acetyltransferases in cellular signaling. Acetylation by p300 of p53 enhances the DNA-binding activity of this important sequence-specific activator (174). A recent striking finding showed that acetylation does not necessarily represent a positive impetus for transcription. Acetylation by *Drosophila* CBP of the *wingless* signaling pathway T-cell transcription factor (TCF) acts as a negative stimulus for signaling flux through this pathway (175). Data for the role of acetylation in directly regulating nuclear receptor function are as yet sparse, although our laboratory has shown that the acetyltransferase activity of PCAF targets zinc finger lysine residues in the DBD of PR (M. Burcin, personal communication). Although the functional consequences of this are as yet unclear, it may be that subtle covalent modifications such as these are important determinants of the association of receptor with its response element, and with coregulators, during transcriptional activation.

D. Chromatin-remodeling proteins

Increasing importance is being attached to recruitment by nuclear receptors of protein complexes that mediate chro-

matin remodeling, a term referring to the regulation of the coherence of the higher order chromatin domains into which nucleosomes are organized (*Section IV.A*). This section will summarize several proteins and protein complexes that have been suggested to be recruited by nuclear receptors to effect chromatin remodeling.

1. The SWI/SNF complex. Particularly well characterized in the process of chromatin remodeling are the products of the *swi/snf* genes. These genes were first identified in yeast on the basis of a genetic screen for genes required for regulation of mating type switching (176, 177). Genetic studies and biochemical purification also indicated that SWI/SNF proteins might form a complex that actively disrupted chromatin. Mutations in histone genes alleviate the requirement for functional SWI/SNF genes in yeast (178). Furthermore, SWI2/SNF2 has intrinsic ATPase activity (179), and purified SWI/SNF complex alters nucleosomal structure *in vitro* in a ATP-dependent manner (180, 181).

A wealth of data has implicated members of the SWI/SNF complex in transcriptional regulation by nuclear receptors. Yoshinaga *et al.* (182) showed that a yeast strain bearing mutations in the *swi1*, *swi2*, and *swi3* genes was incapable of transactivating a reporter gene in the presence of cotransfected GR, whereas a wild-type strain was able to support GR-dependent transactivation. In addition, it was shown that GR coimmunoprecipitated with the SWI/SNF complex (182). Purification of the mammalian homolog of the yeast SWI/SNF complex has identified two genes with a high degree of sequence similarity to *swi2* and *snf2*, named *brahma* (*brm*) and *brahma-related gene 1 (brg-1)* for their similarity to the *Drosophila brahma* gene (183). The products of the human *brm* and *brg-1* genes, hBRM and BRG-1, respectively, are reported to interact with ER in a ligand-dependent manner in a yeast two-hybrid assay (184). In addition, GR recruits the ligand-dependent nucleosomal remodeling activity of the SWI/SNF complex in yeast (185). Fryer and Archer (186) identified the dependence of GR regulation of a stably integrated MMTV promoter upon recruitment of BRG-1-containing complexes. A model for the role of HATs and chromatin-modifying enzymes in facilitating recruitment of a preinitiation complex by liganded receptor is shown in Fig. 4.

2. The TIF-1 proteins. Le Douarin *et al.* (187) have isolated a mouse protein, TIF-1α, which interacts with a RAR-LBD bait in a yeast two-hybrid screen. TIF-1α has been shown to complement RXRγ AF-2 activity in yeast in the presence of 9-*cis*-retinoic acid. Functional interactions in yeast have been demonstrated between TIF-1α and VDR, PR, and ER (188, 189). Paradoxically, however, TIF-1α down-regulates RXRα, RAR, and ER transactivation in mammalian transient transfection assays (187). Furthermore, when fused to a heterologous DBD, TIF-1α represses transcription (190).

Recent data suggest that a family of TIF-1 proteins exists, including TIF-1β and TIF-1γ in addition to TIF-1α (189, 191). This family is defined by an N-terminal domain containing a cysteine-histidine cluster (PHD or plant homeodomain), a RING finger, and a B box finger, domains thought to mediate DNA-protein and protein-protein interactions (192). While Le Douarin *et al.* (190) note that TIF-1β, unlike TIF-1α, does not interact with nuclear receptors, Chang *et al.* (193) have provided evidence that it interacts with GR and C/EBPβ to induce expression of the α1-acid glycoprotein gene. Although their function in nuclear receptor action is unclear, the interactions of TIF-1 family members with heterochromatin-associated proteins indicate a potential role in chromatin modification. TIF-1α has been shown to interact with the heterochromatin-associated proteins mHP1α, MOD1 (HP1β), and MOD2 (HP1γ; 189) which in turn interact with mSNF2-β, the mouse homolog of the *Drosophila* brahma protein (*Section IV.D.1*). Intriguingly, TIF-1α (189) and TIF1β (191) associate with the KRAB (Krüppel-associated box) repression domain, a region conserved in many Krüppel-type zinc finger proteins. A model has been suggested for TIF-1s in transcriptional regulation, in which formation of transcriptionally inactive chromatin domains by TIF-1s effects repression, and ligand-dependent association of TIF-1s with receptors mediates formation of transcriptionally primed chromatin domains. An alternative mode of action for TIF-1s is suggested by the observation that TIF-1α is a protein kinase that targets the basal transcription factors TFIIEα, $TAF_{II}28$, and $TAF_{II}55$ for phosphorylation *in vitro*. (194). Our own data suggest that TIF-1α exists *in vivo* as a component of stable preformed multiprotein complexes of approximately 1 megadalton (MDa) in size (N. J. McKenna, unpublished results).

3. NSD-1. The 280-kDa NSD-1 [nuclear receptor-binding, SET domain-containing protein 1 (63)] contains the evolutionarily conserved SET domain, first identified in the *Drosophila* proteins Su (var), E(z), and Trx (195). Certain SET proteins are

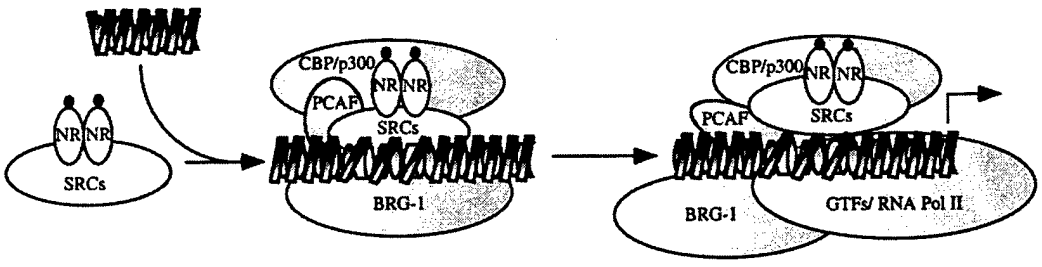

FIG. 4. Two-step model for activation by nuclear receptors at a transcriptionally repressed promoter/enhancer. Liganded receptor recruits SRC family members (denoted SRCs) and other histone acetylases, as well as ATPase-coupled chromatin-modifying enzyme activities to effect local nucleosome disruption around the enhancer/promoter region. Subsequent recruitment of GTFs, RNA Pol II, and other basal factors leads to stabilization of a preinitiation complex. For the sake of clarity, TRAP/DRIP complexes or other less well characterized coregulators have not been represented, nor have direct interactions between receptor and general initiation factors. The reader is referred to the text (*Sections II.A.1* and *II.B.3*) for detailed discussions of these.

thought to be associated with chromatin and commonly function, depending upon the developmental context, either as transcriptional coactivators, as corepressors, or both. For example, while E(z) appears to maintain target genes in a closed chromatin conformation during certain developmental stages, it can act as an activator (196). In contrast, Trx antagonizes the effect of E(z) by maintaining chromatin in a transcriptionally active conformation. These functionally antagonistic properties are also characteristic of NSD-1, which was identified by a two-hybrid screen for RARα-LBD interacting proteins. NSD-1 interacts with the LBD of ER and RARα in the presence of ligand, but its interactions with RXRα and TRα LBDs are reduced in the presence of ligand (63). Moreover, in addition to containing intrinsic activation domains and consensus NR boxes (*Section II.B.2.e*), NSD-1 harbors intrinsic repression domains. While its precise roles are unclear, NSD-1 has been proposed to be a bifunctional coregulator capable of modifying chromatin domains in a developmental stage-specific manner.

E. Corepressors and deacetylation.

Broadly speaking, histone deacetylation opposes the structural incoherence brought to bear upon nucleosomes by histone acetylation (Fig. 3). Extensive genetic studies in yeast have yielded abundant correlative evidence for the global role of hypoacetylation of histones in disabling transcriptional activity and have identified proteins whose mammalian homologs are key factors in transcriptional repression by nuclear receptors.

1. Histone deacetylases and Sin proteins. The product of the yeast *RPD3* gene was isolated as a transcriptional repressor in several independent mutant suppressor screens (197, 198) and was shown to be required for the maximal range of transcriptional efficiency at certain yeast genes. In its absence, both activation and repression of target genes are less efficient (199), indicating a role of RPD-3 in global transcriptional regulation. Rundlett *et al.* (200) demonstrated that a subunit of yeast histone deacetylase activity showed sequence similarity with RPD3. The cloning of a mammalian homolog, histone deacetylase-1 (HD-1/HDAC-1; Ref. 201) established a functional link between histone deacetylation and transcriptional regulation in mammalian cells. HD-1 was isolated by affinity purification using the specific histone deacetylase inhibitor trapoxin. An RPD-3-containing complex, as well as HD-1/HDAC-1, is known to deacetylate core histones *in vivo* (200, 201). Yang *et al.* (202) used a yeast two-hybrid screen to study proteins interacting with the YY-1 transcription factor and identified a cDNA-encoding histone deacetylase-2 (mRPD3/HDAC-2), which bore extensive sequence identity with yeast RPD3. Colinear with the transcriptional regulatory functions of RPD3 are those of another yeast protein, Sin3 (RPD-1), initially identified as a negative regulator of the yeast HO gene (203). The inactivation of the *SIN3* gene, along with other *SIN* genes, was shown to substitute for the requirement of the *SWI5* gene product for *HO* transactivation (204). A model was proposed in which Sin3 effected repression at certain promoters by interaction with specific DNA-binding proteins.

2. Histone deacetylation and nuclear receptor repression. Studies in our laboratory were the first to document the involvement of SIN3 in repression of transcription by nuclear receptors. Nawaz *et al.* (205) demonstrated that SIN3 negatively regulated the transcriptional activity of the PR in a yeast-based promoter system. In addition, yeast strains harboring deletions in the SIN3 gene exhibited increased transactivation of a reporter gene in the presence of liganded PR. A wealth of evidence has since documented the role of mammalian Sin3 homologs and histone deacetylases in repression by nuclear receptors. Anti-NCoR antibodies have been shown to specifically coimmunoprecipitate cellular histone deacetylase activity (206–208). The *in vivo* requirement of Sin3 proteins and histone deacetylase activity by NCoR for repression by TR/RAR heterodimers *in vivo* has been indicated by the ability of anti-mSin3 and anti-mRPD3 antibodies to ablate silencing of a reporter gene by a Gal4DBD-NCoR fusion. Similar results were obtained for a Gal4-TRC′ (Gal4DBD fused to the TRα C-terminal repressor domain) indicating that the repressive effects of TR and NCoR/mSin3-linked histone deacetylase activity are colinear in mammalian cells (206). Laherty *et al.* (209) demonstrated the *in vivo* association of mammalian Sin3 with the two mammalian histone deacetylases, HD-1/HDAC-1 and HDAC-2. In addition, biochemical evidence suggests that Sin3 proteins and histone deacetylases exist in stable preformed complexes in mammalian cells (210, 211). Collectively, these data strongly support the hypothesis that nucleosomal condensation through recruitment of histone deacetylases by corepressors is part of the repertoire by which unliganded type II nuclear receptors inhibit the assembly of a preinitiation complex. An overall model of corepressor/coactivator action (168) envisages unliganded type II receptors maintaining a transcriptionally inactive steady state at the promoter by recruitment of corepressors and their associated histone deacetylase activities. Ligand binding is thought to induce release of corepressors and enable the receptor to recruit PCAF, p300/CBP, and SRC family members to effect local histone acetylation and creation of a transcriptionally permissive environment at the promoter.

3. NURD and Mi-2 ATPase complexes. An interesting footnote to the role of ATPase activity in facilitating transcriptional activation by nuclear receptors (*Section IV.D.1*) is the discovery that ATPase activity may also be harnessed to assist access of nuclear receptor corepressor complexes to promoters (212). The biochemically characterized NURD complex contains a subunit, MTA1, which was shown to contain a region previously identified in NCoR, and immunodepletion of NURD efficiently relieves transcriptional repression by unliganded TR. The coupling by NURD of ATP-dependent nucleosomal remodeling activity to histone deacetylation suggests that nucleosomal disruption may be a key prefatory step in the access of histone deacetylase to its substrate. Wade *et al.* (211) presented similar data with respect to Mi-2, a SNF2-related ATPase (*Section IV.D.1*) that is present in a Sin3/deacetylase complex from *Xenopus laevis*. These results suggest that the acetylation status of histones and their higher order domain structure are not rigidly linked and may be independently manipulated by regulatory proteins.

V. Concluding Remarks

Recent developments in this field have sketched an increasingly complex picture of the functions of nuclear receptor and their associated coregulators. Daunting as it is, however, several strong themes are emerging from the study of this area. A consistently recurrent theme in transcriptional regulation by nuclear receptors is the fluid, multistep nature of the process, in which diverse factors are predicted to have temporally and spatially distinct functions at transcriptionally active promoters. Transcriptional regulation requires the recruitment by receptor of multiple, distinct enzyme activities — acetylases, deacetylases, kinases, ATPases, ligases, and proteases — the concerted action of which, intuitively, must be regulated efficiently to achieve an appropriate transcriptional response to ligand (Table 3). Coregulators are organized into preformed subcomplexes, an arrangement which 1) facilitates their assembly into multiple configurations and 2) makes them readily available to competing pools of transcriptional activators and promoters. Consistent with this level of organization is the notion that efficient activation at different promoters is a function of the assembly of distinct configurations of coregulator complexes at these promoters (172). The requirement of ubiquitin-protein conjugation enzymes for efficient activation by some receptors raises the intriguing possibility that enhancer/promoter clearance of factors may enable the sequential interaction of activated receptor with multiple coregulator complexes. A second theme emerging from the study of coregulators is the multiple layers of control that govern their functional interactions with nuclear receptors. Coregulators appear to be redundant, and no more obvious demonstration than this is provided by the targeted deletion of mSRC-1, a viable phenotype characterized by partial hormone insensitivity and increased, probably compensatory, expression of another SRC family member, mTIF2 (GRIP1/mSRC-2), in many tissues (*Section II.B.2.d*). The functions of nuclear receptor coregulators are governed by factors ranging from tissue-specific patterns of expression (*Section II.B.4.a*) to regulation of their expression by hormone (*Section II.B.2.c*), to environmental stimuli (*II.B.4.d*), to conserved amino acid sequences that determine their physical interaction with liganded receptor (*Section II.B.2.e*). A third theme is the potential of coregulators to act as adaptors to mediate functional interactions of receptors with diverse classes of transcription factors, and integrating receptor-regulated gene networks with a broad spectrum of afferent signals. Implicit in these themes is the prediction that the relative expression level of coactivators and corepressors is an important determinant of an appropriate and graded response to ligand by the target cell.

This review would be incomplete without a brief reference to the clinical and physiological implications of nuclear receptor coregulators. While mechanistic approaches continue to yield essential data, the full impact of these factors on transcriptional biology, and cell biology in general, will be felt as a shift in emphasis from molecular techniques to a more global perspective takes place. Future directions are certain to embrace fundamental questions such as whether (and how) nuclear receptor coregulators contribute to the

TABLE 3. Multifaceted nature of transcriptional regulation by nuclear receptor coregulators: nuclear receptors, through their interactions with coregulators, recruit diverse functional domains and enzyme activities to the promoter to achieve efficient transcriptional regulation *in vivo*

Property	Coregulator	Target	Function	Reference
Activation domain	SRC-1, TIF2/hSRC-2, RAC3/hSRC-3	Basal transcription factors	Stabilization of preinitiation complex	40, 47, 48, 54
Repression domain	NCoR, SMRT, SUNCoR.	Basal transcription factors	Destabilization of preinitiation complex	121, 128
Acetylase	PCAF, CBP/p300, SRC-1, ACTR/hSRC-3	Core histones	Disruption of histone-DNA, histone-histone interactions	53, 165, 167, 171, 172, 173
	p300	p53	Enhances p53 DNA binding	174
	CBP	TCF	Uncouples *Wingless* signaling in *Drosophila*	175
	PCAF	PR	Unknown	–
Deacetylase	HDAC-1/HDAC-2	Core histones, others?	Restoration of DNA-histone, histone-histone interaction	206–209
Protease	Siah2/26S proteosome	NCoR	Protein degradation	142
Ubiquitin ligase	E6-AP	Unknown	Protein degradation	68
ATPase	BRG-1, Trip-1	Chromatin, others?	Domain remodeling during activation	182, 184–186
	SNF2 ATPase	Chromatin, others?	Domain remodeling during repression?	211
Kinase	TIF1α	TFIIEα, $TAF_{II}28$, $TAF_{II}35$	Stabilization/destabilization of target proteins?	194
	ER-associated kinase	?		215
	ANPK (AR-associated kinase)	AR coregulators?		216

etiology of the steroidal cancers of the breast, ovary, uterus, and prostate; their role in the myriad disorders of the endocrine system; and their value as prognostic, diagnostic, or therapeutic targets in such diseases. In addition, targeted deletions of coregulator genes will emerge as a powerful tool in the effort to discern the physiological functions of these factors. We foresee the maturation of these lines of research in the coming years, as this active field strives toward a coherent model of nuclear receptor action.

Acknowledgments

The authors wish to thank Barb Tykal for secretarial assistance. We thank Sophia Y. Tsai, Ming-Jer Tsai, and members of the O'Malley laboratory for valuable discussions during the preparation of the manuscript.

References

1. **Tsai MJ, O'Malley BW** 1994 Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486
2. **Danielian PS, White R, Lees JA, Parker MG** 1992 Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. EMBO J 11:1025–1033
3. **Ham J, Parker MG** 1989 Regulation of gene expression by nuclear hormone receptors. Curr Opin Cell Biol 1:503–511
4. **Godowski PJ, Picard D, Yamamoto KR** 1988 Signal transduction and transcriptional regulation by glucocorticoid receptor-LexA fusion proteins. Science 241:812–816
5. **Dobson AD, Conneely OM, Beattie W, Maxwell BL, Mak P, Tsai MJ, Schrader WT, O'Malley BW** 1989 Mutational analysis of the chicken progesterone receptor. J Biol Chem 264:4207–4211
6. **Housley PR, Sanchez ER, Danielsen M, Ringold GM, Pratt W** 1990 Evidence that the conserved region in the steroid-binding domain of the glucocorticoid receptor is required for both optimal binding of hsp90 and protection from proteolytic cleavage. J Biol Chem 265:12778–12781
7. **Picard D, Yamamoto K** 1987 Two signals mediate hormone-dependent nuclear localisation of the glucocorticoid receptor. EMBO J 6:3333–3340
8. **Roeder RG** 1996 The role of general initiation factors in transcription by RNA polymerase II. Trends Biosci 21:327–335
9. **Zawel L, Reinberg D** 1995 Common themes in assembly and function of eukaryotic transcription complexes. Annu Rev Biochem 64:533–561
10. **Jacq X, Brou C, Lutz Y, Davidson I, Chambon P, Tora L** 1994 Human $TAF_{II}30$ is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. Cell 79:107–117
11. **Mengus G, May M, Jacq X, Staub A, Tora L, Chambon P, Davidson I** 1995 Cloning and characterization of $hTAF_{II}18$, $hTAF_{II}20$ and $hTAF_{II}28$: three subunits of the human transcription factor TFIID. EMBO J 14:1520–1531
12. **Lee S, Hahn S** 1995 Model for binding of transcription factor TFIIB to the TBP-DNA complex. Nature 376:609–612
13. **Ha I, Roberts S, Maldonado E, Sun X, Kim LU, Green M, Reinberg D** 1993 Multiple functional domains of human transcription factor IIB: distinct interactions with two general transcription factors and RNA polymerase II. Genes Dev 7:1021–1032
14. **Koleske AJ, Young RA 1994** An RNA polymerase II holoenzyme responsive to activators. Nature 368:466–469
15. **Chatterjee S, Struhl K** 1995 Connecting a promoter-bound protein to TBP bypasses the need for a transcriptional activation domain. Nature 374:820–822
16. **Schulman IG, Chakravarti D, Juguilon H, Romo A, Evans RM** 1995 Interactions between the retinoid × receptor and a conserved region of the TATA-binding protein mediate hormone-dependent transactivation. Proc Natl Acad Sci USA 92:8288–8292
17. **Sadovsky Y, Webb P, Lopez G, Baxter JD, Fitzpatrick PM, Gizang-Ginsberg E, Cavailles V, Parker MG, Kushner PJ** 1995 Transcriptional activators differ in their responses to overexpression of TATA-box-binding protein. Mol Cell Biol 15:1554–1563
18. **Schwerk C, Klotzbucher M, Sachs M, Ulber V, Klein-Hitpass L** 1995 Identification of a transactivation function in the progesterone receptor that interacts with the $TAF_{II}110$ subunit of the TFIID complex. J Biol Chem 270:21331–21338
19. **McEwan I, Gustafsson J-A** 1997 Interaction of the human androgen receptor transactivation function with the general transcription factor TFIIF. Proc Natl Acad Sci 94:8485–8490
20. **Rochette-Egly C, Adam S, Rossignol M, Egly JM, Chambon P** 1997 Stimulation of RARα activation function AF-1 through binding to the general transcription factor TFIIH and phosphorylation by CDK7. Cell 90:97–107
21. **Blanco JC, Wang IM, Tsai SY, Tsai MJ, O'Malley BW, Jurutka PW, Haussler MR, Ozato K** 1995 Transcription factor TFIIB and the vitamin D receptor cooperatively activate ligand-dependent transcription. Proc Natl Acad Sci USA 92:1535–1539
22. **Ing NH, Beekman JM, Tsai SY, Tsai MJ, O'Malley BW** 1992 Members of the steroid hormone receptor superfamily interact with TFIIB (S300-II). J Biol Chem 267:17617–17623
23. **Conneely OM, Kettelberger DM, Tsai M-J, O'Malley BW** 1989 In: Roy AK, Clark J (eds) Gene Regulation by Steroid Hormones. Springer-Verlag, New York, vol IV:220–231
24. **Meyer ME, Gronemeyer H, Turcotte B, Bocquel MT, Tasset D, Chambon P** 1989 Steroid hormone receptors compete for factors that mediate their enhancer function. Cell 57:433–442
25. **Tora L, White J, Brou C, Tasset D, Webster N, Scheer E, Chambon P** 1989 The human estrogen receptor has two independent nonacidic transcriptional activation functions. Cell 59:477–487
26. **Nagpal S, Saunders M, Kastner P, Durand B, Nakshatri H, Chambon P** 1992 Promoter context- and response element-dependent specificity of the transcriptional activation and modulating functions of retinoic acid receptors. Cell 70:1007–1019
27. **Halachmi S, Marden E, Martin G, MacKay H, Abbondanza C, Brown M** 1994 Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. Science 264:1455–1458
28. **Eggert M, Mows CC, Tripier D, Arnold R, Michel J, Nickel J, Schmidt S, Beato M, Renkawitz R** 1995 A fraction enriched in a novel glucocorticoid receptor-interacting protein stimulates receptor-dependent transcription *in vitro*. J Biol Chem 270:30755–30759
29. **Cavailles V, Dauvois S, Danielian PS, Parker MG** 1994 Interaction of proteins with transcriptionally active estrogen receptors. Proc Natl Acad Sci USA 91:10009–10013
30. **Cavailles V, Dauvois S, L'Horset F, Lopez G, Hoare S, Kushner PJ, Parker MG** 1995 Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. EMBO J 14:3741–3751
31. **Lee CH, Chinpaisal C, Wei LN** 1998 Cloning and characterization of mouse RIP140, a corepressor for nuclear orphan receptor TR2. Mol Cell Biol 18:6745–6755
32. **Li H, Chen JD** 1998 The receptor-associated coactivator 3 activates transcription through CREB-binding protein recruitment and autoregulation. J Biol Chem 273:5948–5954
33. **Onate SA, Tsai SY, Tsai MJ, O'Malley BW** 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354–1357
34. **Wang JC, Stafford JM, Granner DK** 1998 SRC-1 and GRIP1 coactivate transcription with hepatocyte nuclear factor 4. J Biol Chem 273:30847–30850
35. **Zhu Y, Qi C, Calandra C, Rao MS, Reddy JK** 1996 Cloning and identification of mouse steroid receptor coactivator-1 (mSRC-1), as a coactivator of peroxisome proliferator-activated receptor γ. Gene Expr 6:185–195
36. **Jenster G, Spencer T, Burcin M, Tsai SY, Tsai M-J, O'Malley BW** 1997 Steroid receptor induction of gene transcription — a two-step model. Proc Natl Acad Sci USA 94:7879–7884
37. **Lee SK, Kim HJ, Na SY, Kim TS, Choi HS, Im SY, Lee JW** 1998 Steroid receptor coactivator-1 coactivates activating protein-1-mediated transactivations through interaction with the c-*Jun* and c-*Fos* subunits. J Biol Chem 273:16651–16654

38. **Kim HJ, Kim JH, Lee JW** 1998 Steroid receptor coactivator-1 interacts with serum response factor and coactivates serum response element-mediated transactivations. J Biol Chem 273:28564–28567
39. **Na SY, Lee SK, Han SJ, Choi HS, Im SY, Lee JW** 1998 Steroid receptor coactivator-1 interacts with the p50 subunit and coactivates nuclear factor kappaB-mediated transactivations. J Biol Chem 273:10831–10834
40. **Onate SA, Boonyaratanakornkit V, Spencer TE, Tsai SY, Tsai MJ, Edwards DP, O'Malley BW** 1998 The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors. J Biol Chem 273:12101–12108
41. **McInerney EM, Tsai MJ, O'Malley BW, Katzenellenbogen BS** 1996 Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator. Proc Natl Acad Sci USA 93:10069–10073
42. **Ikonen T, Palvimo JJ, Janne OA** 1997 Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor modulates transcriptional activity and is influenced by nuclear receptor coactivators. J Biol Chem 272:29821–29828
43. **Gill RK, Atkins LM, Hollis BW, Bell NH** 1998 Mapping the domains of the interaction of the vitamin D receptor and steroid receptor coactivator-1. Mol Endocrinol 12:57–65
44. **Takeshita A, Yen PM, Misiti S, Cardona GR, Liu Y, Chin WW** 1996 Molecular cloning and properties of a full-length putative thyroid hormone receptor coactivator. Endocrinology 137:3594–3597
45. **Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK, Rosenfeld MG** 1996 A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85:403–414
46. **Hankinson O** 1995 The aryl hydrocarbon receptor complex. Annu Rev Pharmacol Toxicol 35:307–340
47. **Voegel JJ, Heine MJ, Zechel C, Chambon P, Gronemeyer H** 1996 TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. EMBO J 15:3667–3675
48. **Hong H, Kohli K, Trivedi A, Johnson DL, Stallcup MR** 1996 GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors. Proc Natl Acad Sci USA 93:4948–4952
49. **Hong H, Kohli K, Garabedian MJ, Stallcup MR** 1997 GRIP1, a transcriptional coactivator for the AF-2 transactivation domain of steroid, thyroid, retinoid, and vitamin D receptors. Mol Cell Biol 17:2735–2744
50. **Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK, Rosenfeld MG** 1997 The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. Nature 387:677–684
51. **Voegel JJ, Heine MJS, Tini M, Vivat V, Chambon P, Gronemeyer H** 1998 The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways. EMBO J 17:507–519
52. **Walfish PG, Yoganathan T, Yang YF, Hong H, Butt TR, Stallcup MR** 1997 Yeast hormone response element assays detect and characterize GRIP1 coactivator-dependent activation of transcription by thyroid and retinoid nuclear receptors. Proc Natl Acad Sci USA 94:3697–3702
53. **Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, Evans RM** 1997 Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell 90:569–580
54. **Li H, Gomes PJ, Chen JD** 1997 RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. Proc Natl Acad Sci USA 94:8479–8484
55. **Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS** 1997 AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277:965–968
56. **Takeshita A, Cardona GR, Koibuchi N, Suen C-S, Chin WW** 1997 TRAM-1, a novel 160-kDa thyroid hormone receptor activator molecule exhibits distinct properties from steroid receptor coactivator-1. J Biol Chem 272:27629–27634
57. **Suen CS, Berrodin TJ, Mastroeni R, Cheskis BJ, Lyttle CR, Frail DE** 1998 A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. J Biol Chem 273:27645–27653
58. **McKenna NJ, Nawaz Z, Tsai SY, Tsai M-J, O'Malley BW** 1998 Distinct steady state nuclear receptor coregulator complexes exist *in vivo*. Proc Natl Acad Sci USA 95:11697–11702
59. **Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai MJ, O'Malley BW** 1998 Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science 279:1922–1925
60. **Heery DM, Kalkhoven E, Hoare S, Parker MG** 1997 A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 387:733–736
61. **Ding XF, Anderson CM, Ma H, Hong H, Uht RM, Kushner PJ, Stallcup MR** 1998 Nuclear receptor-binding sites of coactivators glucocorticoid receptor interacting protein 1 (GRIP1) and steroid receptor coactivator 1 (SRC-1): multiple motifs with different binding specificities. Mol Endocrinol 12:302–313
62. **Darimont BD, Wagner RL, Apriletti JW, Stallcup MR, Kushner PJ, Baxter JD, Fletterick RJ, Yamamoto KR** 1998 Structure and specificity of nuclear receptor-coactivator interactions. Genes Dev 12:3343–3356
63. **Huang N, vom Baur E, Garnier JM, Lerouge T, Vonesch JL, Lutz Y, Chambon P, Losson R** 1998 Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. EMBO J 17:3398–3412
64. **Feng W, Ribeiro RC, Wagner RL, Nguyen H, Apriletti JW, Fletterick RJ, Baxter JD, Kushner PJ, West BL** 1998 Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors. Science 280:1747–1749
65. **Moras D, Gronemeyer H** 1998 The nuclear receptor ligand-binding domain: structure and function. Curr Opin Cell Biol 10:384–391
66. **Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL** 1998 The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell 95:927–937
67. **Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV** 1998 Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ. Nature 395:137–143
68. **Nawaz Z, Lonard DM, Smith CL, Lev-Lehman E, Tsai SY, Tsai MJ, O'Malley BW** 1999 The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily. Mol Cell Biol 19:1182–1189
69. **Huibregtse JM, Scheffner M, Beaudenon S, Howley PM** 1995 A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. Proc Natl Acad Sci USA 92:5249
70. **Imhof MO, McDonnell DP** 1996 Yeast RSP5 and its human homolog hRPF1 potentiate hormone-dependent activation of transcription by human progesterone and glucocorticoid receptors. Mol Cell Biol 16:2594–2605
71. **Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L, Horwitz KB** 1997 The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. Mol Endocrinol 11:693–705
72. **Powers CA, Mathur M, Raaka BM, Ron D, Samuels HH** 1998 TLS (translocated-in-liposarcoma) is a high-affinity interactor for steroid, thyroid hormone, and retinoid receptors. Mol Endocrinol 12:4–18
73. **Bertolotti A, Lutz Y, Heard DJ, Chambon P, Tora L** 1996 $hTAF_{II}68$, a novel RNA/ssDNA-binding protein with homology to the proto-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. EMBO J 15:5022–5031
74. **Lee JW, Ryan F, Swaffield JC, Johnston SA, Moore DD** 1995 Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. Nature 374:91–94
75. **Rubin DM, Coux O, Wefes I, Hengartner C, Young RA, Goldberg AL, Finley D** 1996 Identification of the GAL4 suppressor Sug1 as a subunit of the yeast 26S proteasome. Nature 379:655–657
76. **Fondell JD, Ge H, Roeder RG** 1996 Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. Proc Natl Acad Sci USA 93:8329–8333
77. **Zhu Y, Qi C, Jain S, Rao MS, Reddy JK** 1997 Isolation and char-

acterization of PBP, a protein that interacts with peroxisome proliferator-activated receptor. J Biol Chem 272:25500–25506
78. **Rachez C, Suldan Z, Ward J, Chang CP, Burakov D, Erdjument-Bromage H, Tempst P, Freedman LP** 1998 A novel protein complex that interacts with the vitamin D3 receptor in a ligand-dependent manner and enhances VDR transactivation in a cell-free system. Genes Dev 12:1787–1800
79. **Yuan CX, Ito M, Fondell JD, Fu ZY, Roeder RG** 1998 The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. Proc Natl Acad Sci USA 95: 7939–7944
80. **Gu W, Malik S, Ito M, Yuan CX, Fondell JD, Zhang X, Martinez E, Qin J, Roeder RG** 1999 A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation. Mol Cell 3:97–108
81. **Kee BL, Arias J, Montminy MR** 1996 Adaptor-mediated recruitment of RNA polymerase II to a signal-dependent activator. J Biol Chem 271:2373–2375
82. **Fondell JD, Guermah M, Malik S, Roeder RG** 1999 Thyroid hormone receptor-associated proteins and general positive cofactors mediate thyroid hormone receptor function in the absence of the TATA box-binding protein-associated factors of TFIID. Proc Natl Acad Sci USA 96:1959–1964
83. **Ge H, Roeder RG** 1994 Purification, cloning, and characterization of a human coactivator, PC4, that mediates transcriptional activation of class II genes. Cell 78:513–523
84. **Kretzschmar M, Kaiser K, Lottspeich F, Meisterernst M** 1994 A novel mediator of class II gene transcription with homology to viral immediate-early transcriptional regulators. Cell 78:525–534
85. **May M, Mengus G, Lavigne AC, Chambon P, Davidson I** 1996 Human $TAF_{II}28$ promotes transcriptional stimulation by activation function 2 of the retinoid × receptors. EMBO J 15:3093–3104
86. **Mengus G, May M, Carre L, Chambon P, Davidson I** 1997 Human $TAF_{II}135$ potentiates transcriptional activation by the AF-2 s of the retinoic acid, vitamin D3, and thyroid hormone receptors in mammalian cells. Genes Dev 11:1381–1395
87. **Lee JW, Choi HS, Gyuris J, Brent R, Moore DD** 1995 Two classes of proteins dependent on either the presence or absence of thyroid hormone for interaction with the thyroid hormone receptor. Mol Endocrinol 9:243–254
88. **Treuter E, Johansson L, Thomsen JS, Wärnmark A, Leers J, Pelto-Huikko M, Sjöberg M, Wright AP, Spyrou G, Gustafsson J** 1999 Competition between thyroid hormone receptor-associated protein (TRAP) 220 and transcriptional intermediary factor (TIF) 2 for binding to nuclear receptors. Implications for the recruitment of TRAP and p160 coactivator complexes. J Biol Chem 274:6667–6677
89. **Baudino TA, Kraichely DM, Jefcoat Jr SC, Winchester SK, Partridge NC, MacDonald PN** 1998 Isolation and characterization of a novel coactivator protein, NCoA-62, involved in vitamin D-mediated transcription. J Biol Chem 273:16434–16441
90. **Kwiatkowski DJ, Short MP** 1994 Tuberous sclerosis. Arch Dermatol 130:348–354
91. **Kandt RS, Haines JL, Smith M, Northrup H, Gardner RJ, Short MP, Dumars K, Roach ES, Steingold S, Wall S, Blanton SH, Flodman P, Kwiatkowski DJ, Jewell A, Weber JL, Roses AD, Pericak-Vance MA** 1992 Linkage of an important gene locus for tuberous sclerosis to a chromosome 16 marker for polycystic kidney disease. Nat Genet 2:37–41
92. **Henry KW, Yuan X, Koszewski NJ, Onda H, Kwiatkowski DJ, Noonan DJ** 1998 Tuberous sclerosis gene 2 product modulates transcription mediated by steroid hormone receptor family members. J Biol Chem 273:20535–20539
93. **Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, Tsai SY, Tsai M-J, O'Malley BW** 1999 A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. Cell 97:17–27
94. **Yeh S, Chang C** 1996 Cloning and characterization of a specific coactivator, ARA-70, for the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 93:5517–5521
95. **Miyamoto H, Yeh S, Wilding G, Chang C** 1998 Promotion of agonist activity of antiandrogens by the androgen receptor coactivator, ARA70, in human prostate cancer DU145 cells. Proc Natl Acad Sci USA 95:7379–7384
96. **Chang KH, Chen YM, Chen TT, Chou WH, Chen PL, Ma YY, Yangfeng TL, Leng XH, Tsai MJ, O'Malley BW, Lee WH** 1997 A thyroid hormone receptor coactivator negatively regulated by the retinoblastoma protein. Proc Natl Acad Sci USA 94:9040–9045
97. **Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM** 1998 A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92:829–839
98. **Boonyaratanakornkit V, Melvin V, Prendergast P, Altmann M, Ronfani L, Bianchi ME, Taraseviciene L, Nordeen SK, Allegretto EA, Edwards DP** 1998 High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding *in vitro* and transcriptional activity in mammalian cells. Mol Cell Biol 18:4471–4487
99. **Kwok RP, Lundblad JR, Chrivia JC, Richards JP, Bachinger HP, Brennan RG, Roberts SG, Green MR, Goodman RH** 1994 Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature 370:223–226
100. **Avantaggiati ML, Ogryzko V, Gardner K, Giordano A, Levine AS, Kelly K** 1997 Recruitment of p300/CBP in p53-dependent signal pathways. Cell 89:1175–1184
101. **Perkins ND, Felzien LK, Betts JC, Leung K, Beach DH, Nabel GJ** 1997 Regulation of NF-κB by cyclin-dependent kinases associated with the p300 coactivator. Science 275:523–527
102. **Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schulman IG, Juguilon H, Montminy M, Evans RM** 1996 Role of CBP/p300 in nuclear receptor signalling. Nature 383:99–103
103. **Fronsdal K, Engedal N, Slagsvold T, Saatcioglu F** 1998 CREB binding protein is a coactivator for the androgen receptor and mediates cross-talk with AP-1. J Biol Chem 273:31853–31859
104. **Smith CL, Onate SA, Tsai MJ, O'Malley BW** 1996 CREB binding protein acts synergistically with steroid receptor coactivator-1 to enhance steroid receptor-dependent transcription. Proc Natl Acad Sci USA 93:8884–8888
105. **Zhou G, Cummings R, Li Y, Mitra S, Wilkinson HA, Elbrecht A, Hermes JD, Schaeffer JM, Smith RG, Moller DE** 1998 Nuclear receptors have distinct affinities for coactivators: characterization by fluorescence resonance energy transfer. Mol Endocrinol 12:1594–1604
106. **Petrij F, Giles RH, Dauwerse HG, Saris JJ, Hennekam RC, Masuno M, Tommerup N, van Ommen GJ, Goodman RH, Peters DJ** 1995 Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature 376:348–351
107. **Eckner R, Ewen ME, Newsome D, Gerdes M, DeCaprio JA, Lawrence JB, Livingston DM** 1994 Molecular cloning and functional analysis of the adenovirus E1A-associated 300 kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev 8:869–884
108. **Yuan W, Condorelli G, Caruso M, Felsani A, Giordano A** 1996 Human p300 protein is a coactivator for the transcription factor MyoD. J Biol Chem 271:9009–9013
109. **Yao TP, Ku G, Zhou N, Scully R, Livingston DM** 1996 The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. Proc Natl Acad Sci USA 93:10626–10631
110. **Hanstein B, Eckner R, DiRenzo J, Halachmi S, Liu H, Searcy B, Kurokawa R, Brown M** 1996 p300 Is a component of an estrogen receptor coactivator complex. Proc Natl Acad Sci USA 93:11540–11545
111. **Yao TP, Oh SP, Fuchs M, Zhou ND, Ch'ng LE, Newsome D, Bronson RT, Li E, Livingston DM, Eckner R** 1998 Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. Cell 93:361–372
112. **Kraus WL, Kadonaga JT** 1998 p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation. Genes Dev 12:331–342
113. **Hudson LG, Santon JB, Glass CK, Gill GN** 1990 Ligand-activated thyroid hormone and retinoic acid receptors inhibit growth factor receptor promoter expression. Cell 62:1165–1175
114. **Glass CK, Lipkin SM, Devary OV, Rosenfeld MG** 1989 Positive and negative regulation of gene transcription by a retinoic acid-thyroid hormone receptor heterodimer. Cell 59:697–708

115. **Forman BM, Samuels HH** 1990 Dimerization among nuclear hormone receptors. New Biol 2:587–594
116. **Baniahmad A, Ha I, Reinberg D, Tsai S, Tsai M-J, O'Malley BW** 1993 Interaction of human thyroid hormone receptor β with transcription factor TFIIB may mediate target gene derepression and activation by thyroid hormone. Proc Natl Acad Sci USA 90:8832–8836
117. **Damm K, Thompson CC, Evans RM** 1989 Protein encoded by v-erbA functions as a thyroid-hormone receptor antagonist. Nature 339:593–597
118. **Baniahmad A, Kohne AC, Renkawitz R** 1992 A transferable silencing domain is present in the thyroid hormone receptor, in the v-erbA oncogene product and in the retinoic acid receptor. EMBO J 11:1015–1023
119. **Casanova J, Helmer E, Selmi-Ruby S, Qi JS, Au-Fliegner M, Desai-Yajnik V, Koudinova N, Yarm F, Raaka BM, Samuels HH** 1994 Functional evidence for ligand-dependent dissociation of thyroid hormone and retinoic acid receptors from an inhibitory cellular factor. Mol Cell Biol 14:5756–5765
120. **Baniahmad A, Leng X, Burris TP, Tsai SY, Tsai MJ, O'Malley BW** 1995 The τ4 activation domain of the thyroid hormone receptor is required for release of a putative corepressor(s) necessary for transcriptional silencing. Mol Cell Biol 15:76–86
121. **Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK, Rosenfeld M** 1995 Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377:397–404
122. **Seol W, Mahon MJ, Lee YK, Moore DD** 1996 Two receptor interacting domains in the nuclear hormone receptor corepressor RIP13/N-CoR. Mol Endocrinol 10:1646–1655
123. **Hollenberg AN, Monden T, Madura JP, Lee K, Wondisford FE** 1996 Function of nuclear co-repressor protein on thyroid hormone response elements is regulated by the receptor A/B domain. J Biol Chem 271:28516–28520
124. **Zamir I, Harding HP, Atkins GB, Horlein A, Glass CK, Rosenfeld MG, Lazar MA** 1996 A nuclear hormone receptor corepressor mediates transcriptional silencing by receptors with distinct repression domains. Mol Cell Biol 16:5458–5465
125. **Shibata H, Nawaz Z, Tsai SY, O'Malley BW, Tsai MJ** 1997 Gene silencing by chicken ovalbumin upstream promoter-transcription factor I (COUP-TFI) is mediated by transcriptional corepressors, nuclear receptor-corepressor (N-CoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT). Mol Endocrinol 11:714–724
126. **Crawford PA, Dorn C, Sadovsky Y, Milbrandt J** 1998 Nuclear receptor DAX-1 recruits nuclear receptor corepressor N-CoR to steroidogenic factor 1. Mol Cell Biol 18:2949–2956
127. **Muscat GE, Burke LJ, Downes M** 1998 The corepressor N-CoR and its variants RIP13a and RIP13Delta1 directly interact with the basal transcription factors TFIIB, $TAF_{II}32$ and $TAF_{II}70$. Nucleic Acids Res 26:2899–2907
128. **Chen JD, Evans RM** 1995 A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377:454–457
129. **Sande S, Privalsky ML** 1996 Identification of TRACs (T3 receptor-associating cofactors), a family of cofactors that associate with, and modulate the activity of, nuclear hormone receptors. Mol Endocrinol 10:813–825
130. **Chen JD, Umesono K, Evans RM** 1996 SMRT isoforms mediate repression and anti-repression of nuclear receptor heterodimers. Proc Natl Acad Sci USA 93:7567–7571
131. **Wong CW, Privalsky ML** 1998 Transcriptional silencing is defined by isoform- and heterodimer-specific interactions between nuclear hormone receptors and corepressors. Mol Cell Biol 18:5724–5733
132. **Tsai S, Collins SJ** 1993 A dominant negative retinoic acid receptor blocks neutrophil differentiation at the promyelocyte stage. Proc Natl Acad Sci USA 90:7153–7157
133. **Yoh SM, Chatterjee VK, Privalsky ML** 1997 Thyroid hormone resistance syndrome manifests as an aberrant interaction between mutant T_3 receptors and transcriptional corepressors. Mol Endocrinol 11:470–480
134. **Tagami T, Madison LD, Nagaya T, Jameson JL** 1997 Nuclear receptor corepressors activate rather than suppress basal transcription of genes that are negatively regulated by thyroid hormone. Mol Cell Biol 17:2642–2648
135. **Dhordain P, Albagli O, Lin RJ, Ansieau S, Quief S, Leutz A, Kerckaert JP, Evans RM, Leprince D** 1997 Corepressor SMRT binds the BTB/POZ repressing domain of the LAZ3/BCL6 oncoprotein. Proc Natl Acad Sci USA 94:10762–10767
136. **Hong SH, David G, Wong CW, Dejean A, Privalsky ML** 1997 SMRT corepressor interacts with PLZF and with the PML-retinoic acid receptor α (RARα) and PLZF-RARα oncoproteins associated with acute promyelocytic leukemia. Proc Natl Acad Sci USA 94: 9028–9033
137. **Lin RJ, Nagy L, Inoue S, Shao W, Miller Jr WH, Evans RM** 1998 Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature 391:811–814
138. **Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, Fanelli M, Ruthardt M, Ferrara FF, Zamir I, Seiser C, Lazar MA, Minucci S, Pelicci PG** 1998 Fusion proteins of the retinoic acid receptor-α recruit histone deacetylase in promyelocytic leukaemia. Nature 391:815–818
139. **Kao HY, Ordentlich P, Koyano-Nakagawa N, Tang Z, Downes M, Kintner CR, Evans RM, Kadesch T** 1998 A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. Genes Dev 12:2269–2277
140. **Baniahmad A, Dressel U, Renkawitz R** 1998 Cell-specific inhibition of retinoic acid receptor-α silencing by the AF2/τc activation domain can be overcome by the corepressor SMRT, but not by N-CoR. Mol Endocrinol 12:504–512
141. **Zamir I, Zhang J, Lazar MA** 1997 Stoichiometric and steric principles governing repression by nuclear hormone receptors. Genes Dev 11:835–846
142. **Zhang J, Guenther MG, Carthew RW, Lazar MA** 1998 Proteasomal regulation of nuclear receptor corepressor-mediated repression. Genes Dev 12:1775–1780
143. **Burris TP, Nawaz Z, Tsai MJ, O'Malley BW** 1995 A nuclear hormone receptor-associated protein that inhibits transactivation by the thyroid hormone and retinoic acid receptors. Proc Natl Acad Sci USA 92:9525–9529
144. **Zamir I, Dawson J, Lavinsky RM, Glass CK, Rosenfeld MG, Lazar MA** 1997 Cloning and characterization of a corepressor and potential component of the nuclear hormone receptor repression complex. Proc Natl Acad Sci USA 94:14400–14405
145. **Xu J, Nawaz Z, Tsai SY, Tsai MJ, O'Malley BW** 1996 The extreme C terminus of progesterone receptor contains a transcriptional repressor domain that functions through a putative corepressor. Proc Natl Acad Sci USA 93:12195–12199
146. **Vegeto E, Allan GF, Schrader WT, Tsai MJ, McDonnell DP, O'Malley BW** 1992 The mechanism of RU486 antagonism is dependent on the conformation of the carboxy-terminal tail of the human progesterone receptor. Cell 69:703–713
147. **Lanz RB, Rusconi S** 1994 A conserved carboxy-terminal subdomain is important for ligand interpretation and transactivation by nuclear receptors. Endocrinology 135:2183–2195
148. **Smith CL, Nawaz Z, O'Malley BW** 1997 Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. Mol Endocrinol 11:657–666
149. **Zhang X, Jeyakumar M, Petukhov S, Bagchi MK** 1998 A nuclear receptor corepressor modulates transcriptional activity of antagonist-occupied steroid hormone receptor. Mol Endocrinol 12:513–524
150. **Lavinsky RM, Jepsen K, Heinzel T, Torchia J, Mullen TM, Schiff R, Del-Rio AL, Ricote M, Ngo S, Gemsch J, Hilsenbeck SG, Osborne CK, Glass CK, Rosenfeld MG, Rose DW** 1998 Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. Proc Natl Acad Sci USA 95:2920–2925
151. **Wagner BL, Norris JD, Knotts TA, Weigel NL, McDonnell DP** 1998 The nuclear corepressors NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor. Mol Cell Biol 18: 1369–1378
152. **Richmond TJ, Finch JT, Rushton B, Rhodes D, Klug A** 1984 Structure of the nucleosome core particle at 7 A resolution. Nature 311:532–537
153. **Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ**

1997 Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 389:251–260
154. **Archer TK, Lefebvre P, Wolford RG, Hager GL** 1992 Transcription factor loading on the MMTV promoter: a bimodal mechanism for promoter activation. Science 255:1573–1576
155. **Mymryk JS, Archer TK** 1995 Dissection of progesterone receptor-mediated chromatin remodeling and transcriptional activation *in vivo*. Genes Dev 9:1366–1376
156. **Wong J, Shi YB, Wolffe AP** 1997 Determinants of chromatin disruption and transcriptional regulation instigated by the thyroid hormone receptor: hormone-regulated chromatin disruption is not sufficient for transcriptional activation. EMBO J 16:3158–3171
157. **Richard-Foy H, Hager GL** 1987 Sequence-specific positioning of nucleosomes over the steroid-inducible MMTV promoter. EMBO J 6:2321–2328
158. **Truss M, Bartsch J, Schelbert A, Hache RJ, Beato M** 1995 Hormone induces binding of receptors and transcription factors to a rearranged nucleosome on the MMTV promoter *in vivo*. EMBO J 14: 1737–1751
159. **Ranjan M, Wong J, Shi YB** 1994 Transcriptional repression of *Xenopus* TRβ gene is mediated by a thyroid hormone response element located near the start site. J Biol Chem 269:24699–24705
160. **Wong J, Shi YB, Wolffe AP** 1995 A role for nucleosome assembly in both silencing and activation of the *Xenopus* TRβA gene by the thyroid hormone receptor. Genes Dev 9:2696–2711
161. **Wolffe AP, Pruss D** 1996 Targeting chromatin disruption: transcription regulators that acetylate histones. Cell 84:817–819
162. **Rhodes D** 1997 The nucleosome core all wrapped up. Nature 389:231, 233
163. **Brownell JE, Zhou J, Ranalli T, Kobayashi R, Edmondson DG, Roth SY, Allis CD** 1996 Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell 84:843–851
164. **Mizzen CA, Yang XJ, Kokubo T, Brownell JE, Bannister AJ, Owen-Hughes T, Workman J, Wang L, Berger SL, Kouzarides T, Nakatani Y, Allis CD** 1996 The $TAF_{II}250$ subunit of TFIID has histone acetyltransferase activity. Cell 87:1261–1270
165. **Yang XJ, Ogryzko VV, Nishikawa J, Howard BH, Nakatani Y** 1996 A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature 382:319–324
166. **vom Baur E, Harbers M, Um SJ, Benecke A, Chambon P, Losson R** 1998 The yeast Ada complex mediates the ligand-dependent activation function AF-2 of retinoid × and estrogen receptors. Genes Dev 12:1278–1289
167. **Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou JX, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai M-J, O'Malley BW** 1997 Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 389:194–198
168. **Blanco JC, Minucci S, Lu J, Yang X-J, Walker K, Chen H, Evans RM, Nakatani Y, Ozato K** 1998 The histone acetylase PCAF is a nuclear receptor coactivator. Genes Dev 12:1638–1651
169. **Ogryzko VV, Kotani T, Zhang X, Schlitz RL, Howard T, Yang XJ, Howard BH, Qin J, Nakatani Y** 1998 Histone-like TAFs within the PCAF histone acetylase complex. Cell 94:35–44
170. **Grant PA, Schieltz D, Pray-Grant MG, Steger DJ, Reese JC, Yates JR, Workman JL** 1998 A subset of TAF_{II}s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. Cell 94:45–53
171. **Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y** 1996 The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 87:953–959
172. **Bannister AJ, Kouzarides T** 1996 The CBP co-activator is a histone acetyltransferase. Nature 384:641–643
173. **Korzus E, Torchia J, Rose DW, Xu L, Kurokawa R, McInerney E, Mullen T-M, Glass CK, Rosenfeld MG** 1998 Transcription factor-specific requirements for coactivators and their acetyltransferase functions. Science 279:703–707
174. **Gu W, Roeder RG** 1997 Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell 90:595–606
175. **Waltzer L, Bienz M** 1998 *Drosophila* CBP represses the transcription factor TCF to antagonize Wingless signalling. Nature 395:521–525
176. **Breeden L, Nasmyth K** 1987 Similarity between cell-cycle genes of budding yeast and fission yeast and the Notch gene of *Drosophila*. Nature 329:651–654
177. **Breeden L, Nasmyth K** 1987 Cell cycle control of the yeast HO gene: cis- and trans-acting regulators. Cell 48:389–397
178. **Winston F, Carlson M** 1992 Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet 8:387–391
179. **Laurent BC, Treich I, Carlson M** 1993 The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. Genes Dev 7:583–591
180. **Cote J, Quinn J, Workman JL, Peterson CL** 1994 Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science 265:53–60
181. **Imbalzano AN, Kwon H, Green MR, Kingston RE** 1994 Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature 370:481–485
182. **Yoshinaga SK, Peterson CL, Herskowitz I, Yamamoto KR** 1992 Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science 258:1598–1604
183. **Muchardt C, Yaniv M** 1993 A human homologue of *Saccharomyces cerevisiae* SNF2/SWI2 and Drosophila *brm* genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J 12: 4279–4290
184. **Ichinose H, Garnier JM, Chambon P, Losson R** 1997 Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. Gene 188:95–100
185. **Ostlund Farrants AK, Blomquist P, Kwon H, Wrange O** 1997 Glucocorticoid receptor-glucocorticoid response element binding stimulates nucleosome disruption by the SWI/SNF complex. Mol Cell Biol 17:895–905
186. **Fryer CJ, Archer TK** 1998 Chromatin remodelling by the glucocorticoid receptor requires the BRG1 complex. Nature 393:88–91
187. **Le Douarin B, Zechel C, Garnier JM, Lutz Y, Tora L, Pierrat P, Heery D, Gronemeyer H, Chambon P, Losson R** 1995 The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-*raf* in the oncogenic protein T18. EMBO J 14:2020–2033
188. **Thenot S, Henriquet C, Rochefort H, Cavailles V** 1997 Differential interaction of nuclear receptors with the putative human transcriptional coactivator hTIF1. J Biol Chem 272:12062–12068
189. **Le Douarin B, Nielsen AL, Garnier JM, Ichinose H, Jeanmougin F, Losson R, Chambon P** 1996 A possible involvement of TIF1α and TIF1β in the epigenetic control of transcription by nuclear receptors. EMBO J 15:6701–6715
190. **Le Douarin B, You J, Nielsen AL, Chambon P, Losson R** 1998 TIF1α: a possible link between KRAB zinc finger proteins and nuclear receptors. J Steroid Biochem Mol Biol 65:43–50
191. **Moosmann P, Georgiev O, Le Douarin B, Bourquin JP, Schaffner W** 1996 Transcriptional repression by RING finger protein TIF1β that interacts with the KRAB repressor domain of KOX1. Nucleic Acids Res 24:4859–4867
192. **Freemont PS** 1993 The RING finger: a novel protein sequence motif related to the zinc finger. Ann NY Acad Sci 684:174–192
193. **Chang CJ, Chen YL, Lee SC** 1998 Coactivator TIF1β interacts with transcription factor C/EBPβ and glucocorticoid receptor to induce α1-acid glycoprotein gene expression. Mol Cell Biol 18:5880–5887
194. **Fraser RA, Heard DJ, Adam S, Lavigne AC, Le Douarin B, Tora L, Losson R, Rochette-Egly C, Chambon P** 1998 The putative cofactor TIF1α is a protein kinase that is hyperphosphorylated upon interaction with liganded nuclear receptors. J Biol Chem 273:16199–16204
195. **Tschiersch B, Hofmann A, Krauss V, Dorn R, Korge G, Reuter G** 1994 The protein encoded by the *Drosophila* position-effect variegation suppressor gene Su(var)3–9 combines domains of antagonistic regulators of homeotic gene complexes. EMBO J 13:3822–3831
196. **LaJeunesse D, Shearn A** 1996 E(z): a polycomb group gene or a trithorax group gene? Development 122:2189–2197
197. **Vidal M, Gaber RF** 1991 RPD3 encodes a second factor required to achieve maximum positive and negative transcriptional states in *Saccharomyces cerevisiae*. Mol Cell Biol 11:6317–6327
198. **Stillman DJ, Dorland S, Yu Y** 1994 Epistasis analysis of suppressor mutations that allow HO expression in the absence of the yeast SW15 transcriptional activator. Genetics 136:781–788

199. **Wolffe AP** 1997 Sinful repression. Nature 387:16–17
200. **Rundlett SE, Carmen AA, Kobayashi R, Bavykin S, Turner BM, Grunstein M** 1996 HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. Proc Natl Acad Sci USA 93:14503–14508
201. **Taunton J, Hassig CA, Schreiber SL** 1996 A mammalian histone deacetylase related to the yeast transcriptional regulator RPD3p. Science 272:408–411
202. **Yang WM, Inouye C, Zeng Y, Bearss D, Seto E** 1996 Transcriptional repression by YY1 is mediated by interaction with a mammalian homolog of the yeast global regulator RPD3. Proc Natl Acad Sci USA 93:12845–12850
203. **Kostriken R, Strathern JN, Klar AJ, Hicks JB, Heffron F** 1983 A site-specific endonuclease essential for mating-type switching in Saccharomyces cerevisiae. Cell 35:167–174
204. **Nasmyth K** 1993 Control of the yeast cell cycle by the CDC28 protein kinase. Curr Opin Celi Biol 5:166–179
205. **Nawaz Z, Baniahmad C, Burris TP, Stillman DJ, O'Malley BW, Tsai MJ** 1994 The yeast SIN3 gene product negatively regulates the activity of the human progesterone receptor and positively regulates the activities of GAL4 and the HAP1 activator. Mol Gen Genet 245:724–733
206. **Heinzel T, Lavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo SD, Davie JR, Seto E, Eisenman RN, Rose DW, Glass CK, Rosenfeld MG** 1997 A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature 387:43–48
207. **Alland L, Muhle R, Hou Jr H, Potes J, Chin L, Schreiber-Agus N, DePinho RA** 1997 Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. Nature 387:49–55
208. **Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, Schreiber SL, Evans RM** 1997 Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell 89:373–380
209. **Laherty CD, Yang WM, Sun JM, Davie JR, Seto E, Eisenman RN** 1997 Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression. Cell 89:349–356
210. **Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP** 1998 Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet 19:187–191
211. **Wade PA, Jones PL, Vermaak D, Wolffe AP** 1998 A multiple subunit Mi-2 histone deacetylase from *Xenopus laevis* cofractionates with an associated Snf2 superfamily ATPase. Curr Biol 8:843–846
212. **Xue Y, Wong J, Moreno GT, Young MK, Cote J, Wang W** 1998 NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Mol Cell 2:851–861
213. **Moilanen AM, Poukka H, Karvonen U, Hakli M, Janne OA, Palvimo JJ** 1998 Identification of a novel RING finger protein as a coregulator in steroid receptor-mediated gene transcription. Mol Cell Biol 18:5128–5139
214. **Moilanen AM, Karvonen U, Poukka H, Yan W, Toppari J, Janne OA, Palvimo JJ** 1999 A testis-specific androgen receptor coregulator that belongs to a novel family of nuclear proteins. J Biol Chem 274:3700–3704
215. **Loria PM, Greene GL,** Characterization of ligand-dependent estrogen receptor-associated protein complex. Program of the Keystone Symposium on the Nuclear Receptor Gene Family, Lake Tahoe, NV, 1998 (Abstract 435)
216. **Moilanen AM, Karvonen U, Poukka H, Janne OA, Palvimo JJ** 1998 Activation of androgen receptor function by a novel nuclear protein kinase. Mol Biol Cell 9:2527–2543

Session OR1: Basic Oral Session Steroid Receptor Co-Activators I

OR1-1

A NOVEL STEROID RECEPTOR COACTIVATOR, SRA, FUNCTIONS AS AN RNA TRANSCRIPT AND IS ASSOCIATED WITH SRC-1.

Rainer B Lanz,*[1] Neil J McKenna,[1] Sergio A Onate,[1] Urs Albrecht,[2] Sophia Y Tsai,[1] Ming-Jer Tsai,[1] Bert W O'Malley.[1] [1]Cell Biology, Baylor College of Medicine, Houston, TX. [2]Biochemistry, Baylor College of Medicine, Houston, TX

Nuclear receptors play critical roles in the regulation of eukaryotic gene expression by coordinating temporally and spatially distinct functions at their target promoters. We report the isolation and functional characterization of a novel transcriptional coactivator, termed Steroid Receptor RNA Activator (SRA). We show that SRA is expressed in a relatively tissue- and cell-specific manner and that multiple isoforms of SRA exist. SRA is selective for the steroid hormone receptors and mediates transactivation via their amino terminal activation function, AF-1. Importantly, we provide functional and mechanistic evidence that SRA acts as an RNA transcript. We show that transfected SRA functions in the presence of cycloheximide and that SRA mutants containing multiple translational stop signals retain their ability to coactivate reporter gene expression. Biochemical fractionation shows that SRA exists in distinct ribonucleoprotein complexes, one of which contains the nuclear receptor coactivator SRC-1. In addition, we show that SRA can be selectively co-immunoprecipitated by steroid receptors or by SRC-1 in Xenopus oocyte extracts that have been injected with *in vitro*-generated RNAs. We are currently analyzing the secondary structure of SRA; we will present conforming data and discuss possible mechanism of SRA transactivation. To our knowledge, SRA is the first eukaryotic transcriptional cofactor to be characterized as a functional RNA transcript.

OR1-2

A REPRESSOR OF ESTROGEN RECEPTOR ACTIVITY (REA) FUNCTIONS AS AN ANTICOACTIVATOR.

P Martini,* K Ekena, R Delage-Mourroux, M Montano, W Harrington, B S Katzenellenbogen. Dept. of Molecular and Integrative Physiology, University of Illinois and College of Medicine, Urbana, IL

We previously reported (Montano et al., Endocrine Society 80th Annual Meeting

McKenna, N.J., Xu, J., Nawaz, Z., Tsai, S.Y., Tsai, M.-J. and O'Malley, B.W. (1999) Nuclear receptor coactivators: multiple enzymes, multiple complexes, multiple functions. *J. Steroid Biochem. Mol. Biol.* 69, 3–12.

To be forwarded upon receipt of reprints.